AR201-14069

NCIC HPV
Sent by: Jodi Burgess
11/12/2002 12:52 PM

To: NCIC HPV
cc:
cc:
Subject: Submission Letter, Test Plan, and Robust Summaries for Anethole

----- Forwarded by Ralph Northrop/DC/USEPA/US on 11/12/02 12:32 PM -----

"Adams, Tim" <tadams@therobertsgroup.net> on 09/03/2002 03:48:19 PM

To: Rtk Chem/DC/USEPA/US@EPA
cc: "'skrixer@chemintox.com'" <skrixer@chemintox.com>
Subject: Submission Letter, Test Plan, and Robust Summaries for Anethole

Dear Ms. Whitman: On behalf of the High Production Flavor and Fragrance High Production Volume Consortia (FFHPVC), I am submitting the submission letter, test plan, and robust summaries for anethole in pdf. file format. If you have any problems with the transmission of the electronic form of these documents, please contact me at any time.

Best regards,
Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

<<Robust Summaries for Anethole .pdf>> <<Submission Letter for Anethole.doc>> <<Test Plan for Anethole .pdf>>

- Robust Summaries for Anethole .pdf
- Submission Letter for Anethole.doc
- Test Plan for Anethole .pdf

The Flavor and Fragrance High Production Volume Consortia
(FFHPVC)
1620 I Street, N.W.
Suite 925
Washington D.C. 20006
Tel. (202)-331-2325 Fax (202)-463-8998

September 2, 2002

Christie Todd Whitman, Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Ms. Whitman:

On behalf or the member companies of the Terpene Consortium, the Flavor and Fragrance High Production Volume Consortia is pleased to submit the Test Plan and Robust Summaries for the substance designated as "Anethole" to the HPV Challenge Program, AR-201. The Terpene Consortium has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public. This submission includes one electronic copy in pdf. format. A hard copy of this submission is available upon request. The EPA registration number for the Terpene Consortium is

Please feel free to contact me with any questions or comments you might have concerning the submission at tadams@therobertsgroup.net, tadams@chemintox.com or 202-331-2325.

Sincerely,
Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

AR201-14069A

The Flavor and Fragrance High Production Volume Consortia

The Terpene Consortium

Test Plan for Anethole (isomer unspecified) and *trans*-Anethole

Anethole (isomer unspecified)	**CAS No. 104-46-1**
***trans*-Anethole**	**CAS No. 4180-23-8**

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The Flavor and Fragrance High Production Volume Chemical Consortia

1620 I Street, NW, Suite 925

Washington, DC 20006

Phone: 202-331-2325

Fax: 202-463-8998

List of Member Companies

ARIZONA CHEMICAL

BASF CORPORATION

BEDOUKIAN RESEARCH, INC.

BOISE CASCADE CORPORATION

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POLAROME INTERNATIONAL INCORPORATED

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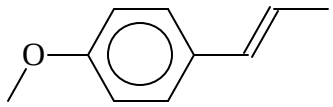
Test Plan for Anethole (isomer unspecified) and *trans*-Anethole

1 IDENTITY OF SUBSTANCES



Anethole (isomer unspecified)

CAS No. 104-46-1

***trans*-Anethole**

CAS No. 4180-23-8

Synonyms:
Anisole, *p*-propenyl-
4-Methoxypropenylbenzene
p-methoxypropenylbenzene
4-Propenylanisole
p-(1-propenyl)anisole
p-Propenylphenyl methyl ether

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The terpene consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for terpene substances under the Chemical Right-to-Know Program. Twenty-one (21) companies are current members of the Terpene Consortium. The Terpene Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

2.2 BACKGROUND INFORMATION

This test plan provides data for two stereochemical forms of anethole. One is the form in which the stereochemistry is unspecified. The Chemical Abstract Registry Number (CAS No. 104-46-1) for this form encompasses both the *cis*- and *trans*- isomer. The other CAS No. (4180-23-8**)** is associated only with the *trans*- isomer. The vast majority of toxicological data is for the *trans*-anethole.

Whether obtained from natural sources (*e.g.*, anise oil or fennel oil), isolated from crude sulfate turpentine, or synthesized, anethole is mainly composed of the *trans*-isomer. *trans*-Anethole is considered by the U.S. Food and Drug Administration (FDA) [21CFR§182.60] and the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavoring substance [Hall and Oser, 1965]. *trans*-

Anethole occurs naturally in more than 20 foods [CIVO-TNO, 2000]. Based on recent and extensive natural occurrence data [CIVO-TNO, 2000] and annual volume of use data [Lucas *et al.*, 1999; Lawrence, 1985] intake of *trans*-anethole from consumption of traditional food approaches 100,000 kg. Anethole is formed in plants as a by-product of terpene synthesis. Although it is a minor (less than 0.5%) [Bauer and Garbe, 1985] constituent of the volatile component of turpentine, significant quantities of anethole are obtained due to the large quantities of turpentine processed.

2.3 STRUCTURAL CLASSIFICATION

Anethole is *p*-methoxypropenylbenzene or *p*-(1-propenyl)anisole. As noted above, whether produced synthetically or derived from natural sources, it occurs mainly as the *trans* isomer. Although anethole is mainly obtained from different synthetic processes [Bauer and Garbe, 1985], it can be isolated directly from crude sulfate turpentine (CST) or can be produced from the CST fraction containing estragole (*p*-methoxyallylbenzene) Isomerization of the double bond of estragole yields a mixture of *cis*- and *trans*-anethole with the *trans*- isomer predominating.

2.4 INDUSTRIAL AND BIOGENIC PRODUCTION

2.4.1 Industrial Production

Industrial sources of anethole have changed to address increasing demand. When demand was low, isolation from essential oils of fennel, anise, and star-anise played a significant role as a commercial source. Increased demand led to isolation from CST or synthetic sources such as anisole. Yields of anethole, its isomer estragole (*p*-methyoxyallylbenzene), the terpene alcohol *alpha*-terpineol, and terpene hydrocarbon caryophyllene are obtained by fractional distillation of CST. Typically, 1-2% of CST is isolated as a mixture of the four substances [Derfer and Traynor, 1992]. Further fractional yield an anethole/caryophyllene mixture accounting for approximately 0.5% of CST. Anethole can be isolated from the mixture by crystallization. The fraction of CST (approximately 1%) containing a mixture of estragole and *alpha*-terpineol can

be treated with potassium hydroxide which results in the isomerization of estragole to yield an 87:13 mixture of *trans*- and *cis*-anethole. The anethole is then isolated by fractional distillation.

Route for the large-scale synthesis of anethole involves treatment of anisole (*p*-methoxybenzene) with propionic acid derivatives or propionaldehyde. In the former synthesis anisole is converted to 4-methoxypropiophenone by a Friedel-Crafts reaction with propionyl chloride [Svadkowskaya *et al*., 1970]. Reaction with propionaldehyde yields the acetal 1,1-bis(4-methoxyphenyl)propane that is subsequently hydrolyzed to anethole and anisole [Bauer and Molleken, 1974].

2.4.2 Biogenic Production

Crude sulfate turpentine (CST) is a complex mixture of C_{10} monoterpene hydrocarbons composed mainly of *alpha*-pinene (60-65%), *beta*-pinene (25-35%) and other monocyclic terpenes including a small amount of anethole (0.04 to 2%) [Millennium Chemicals, 2000]. It has been estimated that the worldwide production of turpentine is approximately 330,000 metric tons of which almost 100,000 metric tons is gum turpentine and the bulk of the remainder (230,000 tons) is sulphate turpentine [National Resources Institute, 1995]. In 1977, the annual United States production of CST and wood turpentine was reported to be 92,750 and 9,150 tons, respectively [McKibben, 1979]. The annual amount of anethole present in CST used in the United States is approximately 460 metric tons (920,000 kg).

Level-three fugacity calculations indicate that the environmental distribution of turpentine and its components is essentially entirely into the air [Mackay *et al*., 1996a, 1996b]. If it were conservatively assumed that through the various industrial processes approximately 2% is lost, the total annual worldwide emission of anethole from turpentine would be 18,400 kg. This can be compared with the biogenic emissions into the air discussed below.

As an important plant terpene by-product, anethole is a component of the earth's atmosphere [Guenther *et al*., 2000]. In determining the impact on the environment of the industrial

production and use anethole, it is also important to examine the impact as a result of emissions from biogenic sources [Guenther *et al*., 2000].

In a recent review of natural emissions of volatile compounds [Guenther *et al*., 1995] it was estimated that 70% of monoterpene flux is accounted for by pines species in North America. The total annual emission of the most common monoterpene constituent of pine (*alpha*-pinene) is approximately 4.5 million metric tons. Given that the ratio of *alpha*-pinene to anethole (0.70 to 0.02) in the volatile component of pine-derived turpentine is approximately 35, it is estimated that biogenic production is approximately 100,000 metric tons annually. Based on total annual global emission of volatile organic compounds (VOC)s [Guenther *et al*., 1995], the percentage *alpha*-pinene in the total emissions of VOCs (mean of 2.6% measured over 3 different forest types), and the pinene/anethole ratio, it can be estimated that the total annual global emissions for anethole is approximately 0.75 million metric tons (*alpha*-pinene emissions, approximately 30 million metric tons).

Based on the above estimate, it can be concluded that total annual atmospheric emission of anethole is predominantly from biogenic sources (750,000,000 kg/year of biogenic emissions *versus* 920,000 kg/year of anthropogenic emissions). The relative contribution from biogenic and industrial sources can be represented by a global emission ratio (GER = biogenic emission/industrial emission). In the case of anethole, the GER would be approximately 1,000, suggesting that biogenic emissions far exceed man-made emissions. As a result, humans are unavoidably exposed to the naturally occurring anethole.

2.5 METABOLISM OF *TRANS*-ANETHOLE

Orally administered *trans*-anethole is rapidly absorbed, undergoes nearly complete metabolism in the liver producing metabolites that are conjugated and then excreted primarily in the urine. Some elimination as CO_2 in expired air also occurs [Fritsch *et al*., 1975; Le Bourhis, 1968, 1970, 1973a; Solheim and Scheline, 1973, 1976]. In the rat, major urinary and intermediary metabolites enter hepatic circulation *via* bile [Solheim and Scheline, 1976].

The pharmacokinetic and metabolic pathways of *trans*-anethole have been well studied in humans, mice and rats and extensively reviewed [Newberne *et al*., 1999]. Three principal pathways of detoxication are followed (*omega*-oxidation, *O*-demethylation, and epoxidation; see Figure 1) and are dependent on dose, animal species, sex, and exposure duration.

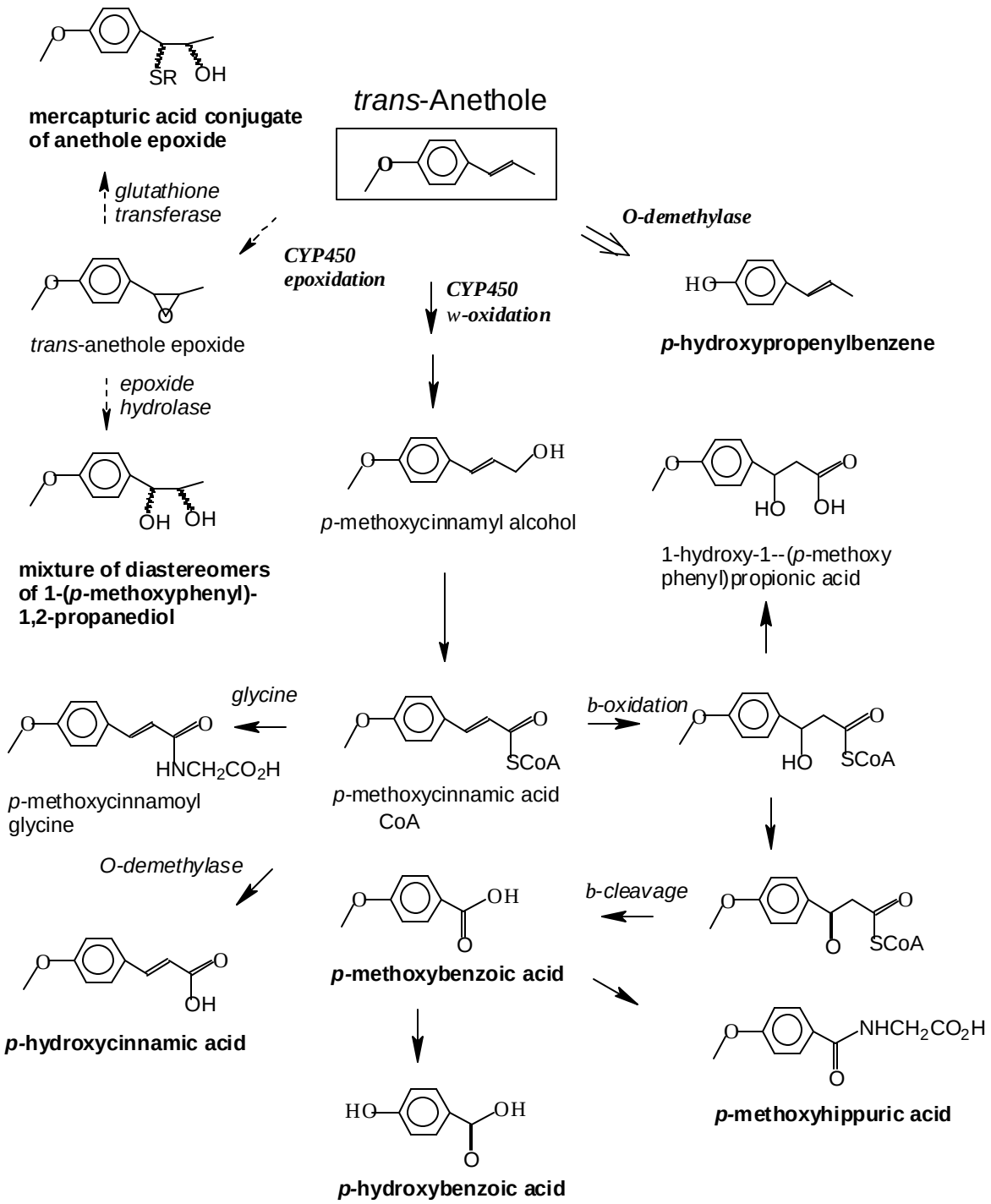
In humans, a single dose of *trans*-anethole was excreted primarily in the urine (60%) following *omega*-oxidation yielding *p*-methoxyhippuric acid (52-56%) and *p*-methoxybenzoic acid within 24 hours (3.5-5%) [Le Bourhis, 1973a; Sangster *et al*., 1987]. Similar results were reported when 3 separate doses of *trans*-anethole were administered. In addition, no dose-dependency on the rate or route of excretion was noted [Caldwell and Sutton, 1988]. About 21% of a 0.05 mg dose of *trans*-anethole was metabolized by *O*-demethylation and expired as CO_2, while approximately 3% of the dose was metabolized to an epoxide [Sangster *et al*., 1987]. The findings of these studies indicated that, when administered to humans at 0.05 to 12 mg/kg bw, *trans*-anethole undergoes metabolic detoxication by *omega*-oxidation and *O*-demethylation.

In mice, an intraperitoneal injection of 50 mg *trans*-anethole/kg bw was excreted in the urine (37%), expired air as CO_2 (47%), and in the feces (less than 2%) within 24 hours [Sangster *et al*., 1984a]. *O*-Demethylation was the critical pathway yielding CO_2 and various urinary metabolites. *omega*-Oxidation accounted for approximately 17.8% of the dose as *p*-methoxyhippuric acid and epoxidation resulted in approximately 6.2% of the dose. When the effect of dose was examined over the range of 0.05 to 1,500 mg/kg bw, a significant shift in metabolism from *O*-demethylation (71.8% at low dose to 34.6% at high dose) to *omega*-oxidation (10.4% at low dose to 43.8% at high dose) and to a lesser extent, epoxidation (1.6% at low dose to 8.8% at high dose) was observed [Sangster *et al*., 1984b]. Also, elimination was slower at higher dose levels (24 hours at low dose and 72 hours at high dose). The effect of dose, sex and pre-feeding also was examined in mice pre-fed *trans*-anethole 62-426 mg/kg bw for 21 days, followed by a single oral gavage dose (equivalent to the pre-feeding level determined from week 3 of the study) [Bounds, 1994; Bounds and Caldwell, 1992, 1996]. The majority of the dose (75-90%) was excreted in the urine of control and treated mice within 24 hours and no effect of dose was noted. At the high dose, pre-fed males tended to excrete more

omega-oxidation metabolites than females. In addition, glycine conjugation of *p*-methoxycinnamic acid decreased with increasing dose and pre-feeding, particularly in females. Accompanying this decrease was a dose-related increase in the formation of glutathione conjugates. Only a slight, but significant, increase in epoxide conjugates was observed in high-dose mice, notably females. The findings of these studies indicate that the major detoxication pathways in mice are *omega*-oxidation and *O*-demethylation.

In rats administered a single oral dose of *trans*-anethole 50 mg/kg bw, *O*-demethylation (41.8%), *omega*-oxidation (12.8%) and epoxidation (14.3%) metabolites were excreted in the urine [Sangster *et al*., 1984a]. Female rats tended to eliminate an intraperitoneal dose of *trans*-anethole 250 mg/kg bw slower than male rats, since after 24 hours, 56% of the dose was recovered in the urine of female rats compared to 71% recovered in male rats [Caldwell, 1991; Caldwell *et al*., 1991]. When the effect of dose was examined over the range of 0.05 to 1,500 mg/kg bw, a significant shift in metabolism from *O*-demethylation (56% at low dose to 32% at high dose) to side-chain *omega*-oxidation (2.6% at low dose to 17.5% at high dose) and to epoxidation (3% at low dose to 18% at high dose) was observed [Sangster *et al*., 1984b]. The effect of dose, sex and pre-feeding also was examined in rats pre-fed *trans*-anethole 100-1,000 mg/kg body for 3 weeks, followed by a single oral gavage dose (equivalent to the pre-feeding level determined from week 3 of the study) [Bounds, 1994; Bounds and Caldwell, 1992, 1996]. In non-pre-fed rats (controls), the rate of elimination of urinary metabolites decreased with increasing dose (80% at low dose *versus* 50% at high dose); whereas, in pre-fed rats, the rate remained essentially constant suggesting that pre-feeding increases the capacity of rats to metabolize and eliminate *trans*-anethole. Overall, detoxication of *trans*-anethole in the rat is dose-dependent and tends to shift from *O*-demethylation to *omega*-oxidation and, more importantly, epoxidation at higher doses.

Figure 1. Metabolism of *trans*-anethole in humans, rats and mice



2.6 SUMMARY FOR CATEGORY ANALYSIS

trans-Anethole, a natural component of the diet, is readily absorbed, metabolized and rapidly excreted *via* the urine as *O*-demethylation and *omega*-oxidation metabolites and, to some extent, epoxidation metabolites. The physiochemical properties and low toxic potential of *trans*-anethole are consistent with its known reactivity and metabolic fate.

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

The melting point of anethole (isomer unspecified) has been reported to be 21.3 °C [CRC, 1995] and for *trans*-anethole 21.4 °C [Merck Index, 1997]. The calculated melting point for *trans*-anethole according to the MPBPWIN program was –0.69 °C [MPBPVP EPI Suite, 2000]. Based on these reported values the melting point of anethole (isomer unspecified) or *trans*-anethole is 21.3-21.4 °C.

3.1.2 Boiling Point

The boiling point of anethole (isomer unspecified) has been reported to be 234 °C [CRC, 1995] and for *trans*-anethole 236 °C [FMA]. The calculated boiling point for *trans*-anethole according to the MPBPWIN program was 217.31 °C [MPBPVP EPI Suite, 2000]. Based on the consistency of these values, the boiling point of anethole (isomer unspecified) or *trans*-anethole is 234-236 °C.

3.1.3 Vapor Pressure

The vapor pressure of anethole (isomer unspecified) has been reported to be 0.041 (5.45 Pa) at 21 °C [Daubert and Danner, 1989]. The calculated vapor pressure of *trans*-anethole has been reported to be 0.05 mm Hg (6.67 Pa) at 20 °C [FMA] and according to the MPBPWIN program was 0.0634 mm Hg (8.45 Pa) at 25 °C [MPBPVP EPI Suite, 2000]. Based on these data the vapor pressure of anethole (isomer unspecified) or *trans*-anethole is approximately 0.05 mm Hg (6.67 Pa) at 20 °C.

3.1.4 n-Octanol/Water Partition Coefficients

Log KOW of *trans*-anethole was calculated resulting in values of 3.39 [KOWWIN EPI Suite, 2000] and 3.11 [Interactive Analysis LogP and LogW Predictor]. The close agreement between calculated values indicated that the log KOW for *trans*-anethole is 3.11-3.39.

3.1.5 Water Solubility

The water solubility of anethole (isomer unspecified) was reported to be 111 mg/L at 25 °C [WSKOW EPI Suite, 2000a]. Water solubility was also calculated for *trans*-anethole resulting in a value of 285.4 mg/L [Interactive Analysis LogP and LogW Predictor] and 139.8 mg/L at 25 °C [WSKOW EPI Suite, 2000b]. Based on these data the water solubility of anethole (isomer unspecified) or *trans*-anethole is approximately 111 mg/L at 25 °C.

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life value for *trans*-anethole has been reported to be 2.015 hours [AOPWIN EPI Suite, 2000]. The fact that anethole contains a reactive allylic hydrogen capable of ready reaction with hydroxyl and peroxy radicals supports the calculated short half-life.

3.2.2 Stability In Water

No hydrolysis is possible for anethole. Anethole (isomer unspecified) or *trans*-anethole is expected to be stable in aqueous solution.

3.2.3 Biodegradation

Anethole (isomer unspecified) demonstrated ready and ultimate biodegradability using a CO2 production test based on OECD Guideline 301B. Biodegradation was 91.0% (90.7-91.2%). [Quest International, 1994].

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay *et al*., 1991a, 1996b] through the EPA EPI suite 2000 program. The input parameters used were molecular weight, vapor pressure (0.41 mm Hg) measured melting point (21.35°C) and boiling point (234°C).

The model predicts that *trans*-anethole is distributed mainly to the soil (69.1%), but also is distributed to water (29.8%) and, to some extent, air (0.53%) and sediment (0.60%).

The significance of these calculations must be evaluated in the context that *trans*-anethole is a product of plant biosynthesis and is, therefore, ubiquitous in the environment. The model does

not account for the influence of biogenic production or chemical reactivity on partitioning in the environment nor does it take into account any biodegradation.

3.2.5 New Testing Required

None.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

Suitable measured and calculated fish LC50s were available for *trans*-anethole. In fathead minnows, the 96-hour LC50 was determined to be 7.690 mg/L with an EC50 of 4.810 mg/L [Broderius *et al*., 1990]. The calculated 96-hour LC50 was reported to be 5.423 mg/L (neutral organics) and 2.433 mg/L (SW) and the 14-day LC50 was reported to be 12.251 mg/L [ECOSAR EPI Suite, 2000].

Given the current database of information, it will not be necessary to perform additional acute fish toxicity tests.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Measured and calculated aquatic invertebrate LC50s were available for *trans*-anethole. In *Daphnia magna*, the 48-hour LC50 was determined to be 6.82 mg/L with a 48-hour EC50 of 4.25 mg/L [Broderius *et al*., 1990]. In addition, calculated values were reported for 48-hour LC50 of 6.397 mg/L and a 16-day EC50 of 0.603 mg/L [ECOSAR EPI Suite, 2000]. A calculated 96-hour LC50 of 0.580 mg/L was reported for mysid shrimp [ECOSAR EPI Suite, 2000].

Given the current database of information, it will not be necessary to perform additional acute aquatic invertebrate toxicity tests.

3.3.3 Acute Toxicity to Aquatic Plants

Measured and calculated values for aquatic plants are available for *trans*-anethole. In green algae, a 96-hour IC50 of 9.571 mg/L was determined [Broderius *et al*., 1990]. A calculated 96-hour EC50 of 4.332 mg/L was reported for green algae [ECOSAR EPI Suite, 2000].

Given the current database of information, it will not be necessary to perform additional acute aquatic plant toxicity tests.

3.3.4 New Testing Required

None.

3.4 HUMAN HEALTH TOXICITY

3.4.1 Acute Toxicity

In rats, mice and guinea pigs, anethole (isomer unspecified and *trans*-) showed low acute toxicity with oral LD50s of 2,090-3,200 mg/kg bw for rats, 3,050-5,000 mg/kg bw for mice, and 2,160 mg/kg bw for guinea pigs, and intraperitoneal LD50s of 703-900 mg/kg bw for rats and 650-1,410 mg/kg bw for mice [Caujolle and Meynier, 1958; Jenner *et al*., 1964; Boisser *et al*., 1967; Borriston Laboratories Inc., 1984].

Mild liver lesions were reported in male and female rats gavaged with anethole 695 mg/kg bw/day for 4 days [Taylor *et al*., 1964].

Given the studies available, additional acute toxicity tests in mammals are not recommended.

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

Anethole (*trans*- and isomer unspecified) was tested extensively in various *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537, or TA1538) at concentrations of up to 600 micrograms/plate in the presence or absence of metabolic activation. *Salmonella typhimurium* strains TA98, TA1535, TA1537, and TA1538 consistently produced negative results [Hsia *et al*., 1979; Nestmann *et al*., 1980; Sekizawa and Shibamoto, 1982; To *et al*., 1982; Mortelmans *et al*., 1986; Heck *et al*., 1989]. Mixed results were reported with *Salmonella typhimurium* strain TA100 showing positive results in the presence of S9 [Sekizawa and Shibamoto, 1982] or S13 [Swanson *et al*., 1979] and negative results in the presence or absence of a metabolic activation system [Hsia *et al*., 1979; Nestmann *et al*., 1980; To *et al*., 1982; Mortelmans *et al*., 1986; Heck *et al*., 1989; Gorelick, 1995].

In a supplementary study, the cofactor 3'-phosphadenosine-5'-phophosulfate (PAPS) was added and significantly increased the mutagenic activity of *trans*-anethole in *Salmonella*

typhimurium strain TA1535 [To *et al*., 1982]; however, this positive finding was not repeated in a more recent study by Gorelick [1995].

Anethole (isomer unspecified) did not induce revertants in the *Escherichia coli* WP2 *uvrA* reversion assay when tested at concentrations of up to 600 micrograms/plate and produced negative results in the *Bacillus subtilis* DNA repair test at a concentration of 10 mg/disk [Sekizawa and Shibamoto, 1982].

trans-Anethole did not produce a response when tested in *Saccharomyces cerevisiae* strains D7 or XV185-14C in the absence of metabolic activation [Nestmann and Lee, 1983].

In rat hepatocytes, anethole (isomer unspecified) did not induce unscheduled DNA synthesis (UDS) when tested at concentrations up to 0.01 M [Heck *et al*., 1989; Marshall *et al*., 1989; Howes *et al*., 1990; Caldwell *et al*., 1992; Marshall and Caldwell, 1996]. In one study with rat hepatocytes, *trans*-anethole showed a very slight response in the UDS assay at a concentration of 0.001 M but showed no response at lower concentrations and was cytotoxic at 0.01 M [Muller *et al*., 1994].

No significant increase in mutant frequency was reported when up to 72 micrograms/ml of *trans*-anethole was tested in L5178Y/TK+/- mouse lymphoma cells in the absence of S9; however, in the presence of S9, *trans*-anethole produced a concentration-dependent increase in mutant frequency paralleled by a decrease in total growth [Gorelick, 1995]. Similar results were reported by Heck *et al*. [1989].

trans-Anethole produced no significant increase in chromosomal aberrations in Chinese hamster ovary (CHO) cells with or without metabolic activation at concentrations of up to 0.2 microliters/ml [Gorelick, 1995].

3.4.2.2 In vivo

Anethole (*trans*- and isomer unspecified) was tested *in vivo* in rats and mice for its potential to affect DNA. Unscheduled DNA synthesis (UDS) was not reported in the hepatocytes of rats

gavaged with *trans*-anethole up to 500 mg/kg bw [Marshall and Caldwell, 1996]. Intraperitoneal injections of anethole up to 10 mg/mouse produced only low levels of DNA adducts in adult and newborn mice [Phillips *et al*., 1984; Randerath *et al*., 1984].

When tested in the micronucleus assay, anethole (isomer unspecified) at doses of up to 1,000 mg/kg bw/day over 7 days did not increase the frequency of micronuclei or affect the ratio of polychromatic erythrocytes to normochromatic erythrocytes in the femoral cells of mice [Al-Harbi *et al*., 1995]. It was concluded that anethole (isomer unspecified) was non-clastogenic.

3.4.2.3 Conclusions

The genotoxicity database on anethole (*trans*- and isomer unspecified) shows no mutagenic potential in the Ames assay. In cytogenetic assays, there is no evidence of a genotoxic potential *in vitro*. In whole animals, the genotoxicity results for anethole showed no micronuclei induction in mice, no UDS response in rats, and limited potential to form DNA adducts in mice. Based on these results no additional genotoxicity tests are recommended.

3.4.3 Repeat Dose Toxicity

Numerous short- and long-term rodent studies have been conducted to evaluate the safety of *trans*-anethole because of its important use as a flavoring substance. Most of the studies conducted were comprehensive, but some also focused on potential hepatic effects due to the results of metabolism studies that indicated that anethole might be a hepatotoxin in rats.

3.4.3.1 *Subacute Studies*

In a preliminary dose range-finding study, groups of 5 rats/sex were fed *trans*-anethole up to 1,200 mg/kg bw/day in the diet for a period of 28 days [Minnema, 1997a]. No notable treatment-related findings were reported at doses up to 300 mg/kg bw/day, although decreased feed consumption was noted in the early part of the study. At the higher doses, in addition to decreased body weight and feed consumption (due to poor palatability of diet), some hepatic effects, as indicated by serum biochemistry results and microscopic examination, were reported.

In a preliminary dose range-finding study, groups of 5 mice/sex were fed *trans*-anethole up to 500 mg/kg bw/day in the diet for a period of 28 days [Minnema, 1997b]. Decreased feed consumption seen at doses of 120 mg/kg bw/day and higher were associated with the poor palatability of the diet. At doses of 240 mg/kg bw/day and above, some mice stopped eating, morality increased (greater than 40%), and decreased body weights were reported, particularly in male mice. No treatment-related histomorphological changes were observed in the liver at any dose. The results from this study appear to be related to the poor palatability of *trans*-anethole in the diet and compromised food intake.

3.4.3.1.1 Special Studies on Immunosuppression

Three studies in mice were conducted to assess the immunosuppressive potential of anethole in mice.

Groups of 20 female mice were gavaged with *trans*-anethole up to 750 mg/kg bw/day for 5 days [IIT Research Institute, 1995a]. On the third day of treatment, mice were also injected intravenously with *Listeria monocytogenes*. No statistically significant differences in mortality or time to death in treated mice compared to controls. *trans*-Anethole did not affect the ability of mice to withstand a *Listeria monocytogenes* challenge.

Groups of 10 female mice were gavaged with *trans*-anethole up to 750 mg/kg bw/day for 5 days [IIT Research Institute, 1995b]. Four days prior to *trans*-anethole treatment, mice were injected intraperitoneally with sheep red blood cells (SRBC) and again after the 5 days of *trans*-anethole treatment. Four days after last intraperitoneal injection, mice were killed and spleens were removed. Plaque-forming cells (PFC) were determined from diluted spleen cells. Absolute thymus weight was significantly decreased in high-dose mice. No other effects were noted. The results of this study indicated that *trans*-anethole did not affect the ability of mice to generate antibody plaque-forming cells following immunization with sheep red blood cells.

In another similar study, groups of 8 male mice were gavaged with anethole 875 mg/kg bw/day for 11 days [Borriston Laboratories, Inc., 1982]. On the third day of treatment, mice were

intraperitoneally injected with 0.3 ml 25% SRBC. On day 12 of the study, the mice were killed and the spleen, thymus and adrenals were removed and weighed. Serum was also isolated and tested for hemagglutinating activity to SRBC. There were no differences in spleen, thymus and adrenal organ weights or in the agglutination scores and calculated antibody index when compared with control values. Anethole was not immunosuppressive in this assay.

3.4.3.1.2 Special Studies on Enzyme Induction

The findings from metabolism and repeat-dose studies indicated that *trans*-anethole might have hepatotoxic effects in the rat, which resulted in examination of its potential to be an enzyme inducer in the liver.

Groups of 7 female rats were gavaged with anethole up to 300 mg/kg bw/day in corn oil for 4 days [Wenk, 1994]. On the fifth day, body weights were taken, rats were killed and livers were removed and homogenized. The homogenate was centrifuged and the supernatant (S9) was used to determine P450 and P448 activity. Enzyme activity was determined using 3 assays: *p*-nitroanisole *O*-demethylation (PNAS), 7-ethoxycoumarin *O*-deethylation (7EC), and ethoxyresorufin *O*-deethylation (EROD). There were no statistically significant differences in body weight or absolute and relative liver weight in treated rats compared to controls. The activities were statistically significant for PNAS and EROD at 300 mg/kg bw/day. In this assay, anethole induced cytochrome P450 and P448 hepatic activity in rats.

Groups of 24 female rats were injected intraperitoneally with *trans*-anethole 300 mg/kg bw/day for 7 days [Reed and Caldwell, 1992a] (No robust summaries were prepared for related biochemical studies in Reed, 1994). Twenty-four hours following last injection, rats were killed, livers were removed and weighed, and hepatic microsomes were prepared. Cytochrome P450 activity was determined using 7-ethoxycoumarin *O*-deethylase. There was a significant (p less than or equal to 0.05) increase in relative liver weights, microsomal protein (18% increase) and in microsomal cytochrome P450 (45% increase) in anethole-treated rats compared to vehicle controls. The authors concluded that *trans*-anethole has a modest enzyme-inducing effect on rat liver.

To further study this effect, groups of 8 rats/sex were fed up to 1.0% *trans*-anethole in the diet for 21 days [Reed and Caldwell, 1992a] (No robust summaries were prepared for related biochemical studies in Reed and Caldwell, 1992b and Reed, 1994). Additional groups of 5 rats/sex were treated similarly, but were allowed to resume the untreated basal diet for 14 days after the 21-day treatment period. Rats were killed, livers were removed and weighed, and hepatic microsomes were prepared. Hepatic microsomal protein and cytochrome P450 levels were determined using cytochrome C reductase, ethoxycoumarin *O*-deethylase, ethoxy and pentoxyresurufin *O*-dealkylase activities. For the rats killed on day 22, significant (p less than or equal to 0.05) increases in relative liver weight were reported in females and males at the two highest doses. Mean protein levels and cytochrome P450 content were significant (p less than or equal to 0.05) at all doses in treated females and at the two highest doses in males. For the rats undergoing a 14-day recovery period, there were no significant differences to controls with the exception of one (considered to be anomalous and related to the lack of sensitivity of the assay) finding of increased cytochrome P450 activity in females fed 0.25% *trans*-anethole. The authors concluded that *trans*-anethole has a modest enzyme-inducing effect on rat liver and noted that female rats tend to be more sensitive. In addition, these effects were reversible when anethole exposure was terminated.

In a supplementary study to determine whether biochemical changes are associated with cell proliferation, groups of 5 rats/sex also were fed up to 1.0% *trans*-anethole in the diet for 21 days [Reed and Caldwell, 1992a] (No robust summaries were prepared for related biochemical studies in Reed and Caldwell, 1992b and Reed, 1994). For the last 3 days of *trans*-anethole exposure, 3 rats/sex/group were given 20 micrograms 5-bromo-2'-deoxyuridine (BrdU) subcutaneous *via* osmotic mini-pumps. Rats were killed and livers were removed. Liver sections were taken and treated with a murine anti-BrdU mAb plus a peroxidase-conjugated second antibody. Preliminary data indicate that liver sections from female rats fed the 0.5% *trans*-anethole diet contain higher numbers of labeled cells than control or rats fed 0.25% *trans*-anethole diet. Conversely, high-dose female rats appear to have fewer labeled cells than the other dose groups. No significant effects reported for males.

Enzyme induction was also studied in mice. Groups of 24 mice/sex were fed 0, 0.1, 0.25, 0.5 or 1.0% *trans*-anethole in the diet for 22 days [Reed and Caldwell, 1993]. Mice in the 1.0% group were terminated prematurely due to severe weight loss. The remaining mice were killed, livers were removed and weighed, and hepatic microsomes were prepared. The diet was unpalatable to the mice resulting in decreased body weight at 0.25 and 0.5%. Relative liver weights were significantly (p less than or equal to 0.005) increased at the two lowest doses, but not at 0.5%. Microsomal protein was significantly (p less than or equal to 0.005 and p less than or equal to 0.05) increased in males given 0.25 and 0.5. Cytochrome P450 was significantly (p less than or equal to 0.005 and p less than or equal to 0.05) increased in males of the 0.5% group and in females of the 0.25 and 0.5% groups. Since caloric restriction is known to induce hepatic cytochrome P450, a similar study was conducted using 0.5% *trans*-anethole but restricted the dietary intake of control mice to that consumed by the treated mice. The comparison of microsomal cytochrome P450 content in these mice still showed a significant (p less than or equal to 0.05) increase over controls. The authors concluded that *trans*-anethole has a modest enzyme-inducing effect on mouse liver.

3.4.3.2 Subchronic Studies

The only effect reported in rats fed 10,000 ppm anethole in the diet for 15 weeks were slight hydropic microscopic changes of hepatocytes in male rats [Hagan *et al*., 1967].

In a series of studies with newborn mice, the authors examined the potential of *trans*-anethole to produce hepatomas when administered through various routes of exposure at an early age. *trans*-Anethole consistently showed no hepatocarcinogenic activity when administered to mice prior to weaning, as described below.

Four-day-old mice were gavaged with *trans*-anethole 0, 2.5 or 5 micromol/kg bw twice weekly for a total of 10 times and were killed between 11 and 14 months of age [Miller *et al*., 1983]. No statistically significant change in the percent of hepatoma-bearing mice, average number or hepatomas/mouse, or number of mice with lung adenomas compared to control values was reported.

Male mice were intraperitoneally injected at 1, 8, 15 and 22 days of age with *trans*-anethole resulting in a total dose of 4.75 micromol [Miller *et al*., 1983]. Some mice were examined by laparotomy at 13 months and those surviving were killed at 18 months of age and examined for induction of hepatomas. No statistically significant change in the percent of hepatoma-bearing mice, or average number of hepatomas/mouse compared to control values was reported.

Another group of male mice were intraperitoneally injected at 1, 8, 15 and 22 days of age with *trans*-anethole resulting in a total dose of 9.45 micromol [Miller *et al*., 1983]. Mice were weaned at 22 days of age, killed at 12 months of age and examined for induction of hepatomas. No statistically significant change in the percent of hepatoma-bearing mice, average number of hepatomas/mouse, or number of mice with lung adenomas compared to control values was reported.

In a separate experiment, female mice were given *trans*-anethole 1 micromol/kg bw by intraperitoneal injection, twice/week for a total of 24 injections and after 8 months were examined for the development of lung adenomas [Miller *et al*., 1983]. No statistically significant change in the percent of mice with lung adenomas or the average number of adenomas/mouse compared to control values was reported. *trans*-Anethole showed no pulmonary carcinogenic activity when administered to mice over 12 weeks.

Comprehensive, GLP-compliant 90-day studies have been conducted in rats and mice [Minnema, 1997c; Minnema, 1997d]. In rats fed *trans*-anethole up to 900 mg/kg bw/day *via* the diet, body weights, feed consumption, and feed efficiency were decreased in males fed 300 mg/kg bw/day or more and in females fed 600 mg/kg bw/day or more. The authors attributed these effects to the poor palatability of the treated diet. Relative liver weights showed statistically significant increases in rats fed 300 mg/kg bw/day or more. Additional effects reported included centrilobular to diffuse hepatocellular hypertrophy in (males at greater than or equal to 300 mg/kg bw/day; females at greater than or equal to 600 mg/kg bw/day), minimal to slight single cell hepatocellular necrosis associated with perivascular inflammatory infiltrate (males at greater than or equal to 600 mg/kg bw/day; females at greater than or equal to 900 mg/kg bw/day),

increased blood *gamma*-glutamyltranferase (males at greater than or equal to 900 mg/kg bw/day; females at greater than or equal to 600 mg/kg bw/day), and increased alanine and aspartate aminotransferase (females at greater than or equal to 900 mg/kg bw/day). These latter effects were considered to be adaptive physiological responses associated with the enzyme induction properties of *trans*-anethole (see section 3.4.3.1.2), rather than adverse effects. Based on the observed necrosis in males and increased levels of *gamma*-glutamyltranferase in females, the no observable adverse effect level (NOAEL) was reported to be 300 mg/kg bw/day.

Similarly, mice were fed up to 240 mg *trans*-anethole/kg bw/day *via* the diet [Minnema, 1997c]. Severe loss of body weight and dehydration were reported mainly at doses of greater than or equal to 120 mg/kg bw/day and were attributed to inanition syndrome (starved mouse syndrome) resulting from the poor palatability of the diet and reduced food intake. Other effects reported included liver glycogen depletion (males at greater than or equal to 30 mg/kg bw/day; females at greater than or equal to 60 mg/kg bw/day), decreased mean cell volume (males at greater than or equal to 120 mg/kg bw/day), decreased mean cell hemoglobin (males at greater than or equal to 120 mg/kg bw/day), reduced cellularity of the spleen (males at 240 mg/kg bw/day), delayed kidney development (males at 240 mg/kg bw/day), increased absolute and relative liver weights (males at greater than or equal to 30 mg/kg bw/day), increased relative thyroid weight (males at greater than or equal to 30 mg/kg bw/day), decreased absolute spleen weight (males at greater than or equal to 60 mg/kg bw/day), decreased relative (to brain) spleen weight (males at greater than or equal to 60 mg/kg bw/day), decreased absolute and relative (to brain) kidney weights (males at greater than or equal to 120 mg/kg bw/day), increased absolute and relative adrenal weights (males at greater than or equal to 60 mg/kg bw/day), decreased absolute heart and adrenal weights (females at 240 mg/kg bw/day), increased incidence of centrilobular hepatocellular hypertrophy (males at greater than or equal to 60 mg/kg bw/day), and increased serum alkaline phosphatase (males at greater than or equal to 120 mg/kg bw/day). As in the rat, the enlarged livers, increased liver weight, and increased incidence of centrilobular hepatocellular hypertrophy were considered to be adaptive physiological

responses to the enzyme inducing effect of *trans*-anethole. Increased serum alkaline phosphatase was considered also to be an adaptive response, or related to the reduced feed intake. The decreased values for mean cell volume and mean cell hemoglobin were not accompanied by significant differences in mean erythrocyte count, hemoglobin or hematocrit. In addition, the decrease was of low magnitude and therefore, the changes were considered incidental. The authors determined the NOAEL to be 240 mg/kg bw/day based on the lack of treatment-related adverse effects. An independent histopathological evaluation on the livers of the rats and mice from the 90-day studies concurred with these conclusions [Newberne, 1997] (no robust summary prepared).

3.4.3.3 Chronic Studies

No effects were reported in rats fed 2,500 ppm anethole for one year [Hagan *et al*., 1967].

Groups of rats were fed up to 1.0% *trans*-anethole in the diet (approximately up to 400 and 550 mg/kg bw/day for males and females, respectively) for up to 177 weeks [Truhaut *et al*., 1989]. An additional group of 26 rats/sex was fed 1% *trans*-anethole until week 54 and then received basal diet only until the end of the study. Between weeks 42-45, most rats showed signs of sialodacryoadenitis resulting in transient retardation of body weight gain. All treated groups showed lower body weight gains. The reversal group showed no difference in body weight gain compared to controls by the end of the study. Mortality was increased in high-dose females and reduced adiposity was reported in high-dose rats, particularly males. No effect on hematological parameters was reported. Notable non-neoplastic effects on the liver included sinusoidal dilatation (at 0.5 and 1%); nodular hyperplasia (at 0.5 and 1% in males and 1% in females); and hepatocytic hypertrophy (at 0.5 and 1% in females). The only statistically significant finding in neoplastic lesions was an increase in the incidence of liver tumors in 1% females, but the authors noted that the increased incidence of hepatocellular carcinomas reported in high-dose females were "late onset", had no effect on longevity and was still within the range of historical controls. The reduced adiposity was considered to be an indirect effect of the poor palatability of the treated diet and decreased feed consumption. The authors

determined a NOAEL of 100 mg/kg bw/day for males based on nodular hyperplasia and 120 mg/kg bw/day in females based on sinusoidal dilatation and hepatocellular hypertrophy and concluded that there was insufficient evidence to conclude that *trans*-anethole is a human carcinogenic risk. An independent group of pathologists re-evaluated the pathology data and concurred with the conclusion [Newberne, 1989] (no robust summary prepared).

Female mice were fed 0.46% trans-anethole in the diet either with or without concurrent exposure to 0.05% phenobarbital in drinking water [Miller *et al*., 1983]. Anethole exposure was stopped at 12 months. Mice were killed after 18 months and examined for induction of hepatomas. There was no statistically significant change in the average number of hepatomas/mouse compared to control values regardless of exposure to phenobarbital.

Given the numerous and comprehensive repeat-dose studies available on anethole, further testing with subacute, subchronic, and chronic protocols is not recommended.

3.4.4 Reproductive Toxicity

Anethole was tested for its potential reproductive toxicity in a comprehensive 4-generation rat study [Le Bourhis, 1973b]. In this study, groups of male and female rats (F_0) were fed 0 or 1% anethole in the diet (approximately 600-1,500 mg/kg bw/day) prior to mating, during the 15-day mating period, and during gestation and lactation. Offspring (F_1) were used for propagating the next generation and were raised on the same dietary treatment as their parents. A similar procedure was followed to obtain the 3rd and 4th generations (F_2 and F_3). The only notable effect was reduced body weight gain and body weights coinciding with reduced feed intake in rats fed 1% anethole. There was no effect on reproductive performance over 4 generations. The reduced palatability of the diet was considered to be responsible for the lower body weight gain and body weights of the rats receiving anethole.

To ascertain the effect of palatability on the effects reported in the 4-generation study, a cross-fostering experiment was conducted using groups of control and treated F_1 females (from the 4-generation study and receiving 1% anethole in the diet) mated with control F_1 males (from the 4-

generation study) [Le Bourhis, 1973b]. Litters born from treated females were exchanged with litters from control females at birth and reared by the new dams. No significant difference in body weights of pups from those nursed by mothers of the same group, regardless from which group they were born, was reported and final body weights of pups born from treated dams but raised by control dams regained normal values by day 28. The results indicated that postnatal growth is not directly affected by anethole exposure, but is a result of the nutritional status of the dams.

Given the comprehensiveness of this study and the concurrence with the results of the developmental study described in section 3.4.5, no further testing on the possible reproductive toxicity of anethole is recommended.

3.4.5 Teratogenicity/Developmental Toxicity

In a developmental and reproductive screening test [Argus Research Laboratories, 1992], groups of female rats were gavaged with anethole 0, 35, 175, or 350 mg/kg bw/day in corn oil for 7 days prior to co-habitation with male rats until day 4 of lactation. Similar to the 4-generation study reported in section 3.4.4, the only notable effects were reduced mean body weights and decreased feed consumption in high-dose rats. These effects were seen to some extent in rats gavaged with anethole 175 mg/kg bw/day, but only reached statistical significance in the early part of the study. At the high dose (350 mg/kg bw/day), the number of liveborn pups was significantly decreased, the number of stillborn pups was significantly increased, the number of pups dying on day 1 and days 2-4 was significantly increased, the viability index (number of live pups on postpartum day 4/number of liveborn pups on postpartum day 1) was significantly decreased, the number of surviving pups/litter on postpartum day 4 was significantly decreased, the live litter size on postpartum day 4 was significantly decreased, and pup weight/litter on postpartum day 1 was significantly decreased compared to controls. No anomalies and no other effects were reported. The authors determined the maternal and developmental NOAEL to be 35 and 175 mg/kg bw/day, respectively, and the maternal and developmental lowest observable adverse effect level (LOAEL) to be 175 and 350 mg/kg

bw/day, respectively. Anethole did not cause any effects on the rat fetus at doses below those causing maternal toxicity (reduced body weight and feed consumption).

No additional testing is recommended given the adequacy of this study.

3.4.6 New Testing Required

None.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 4180-23-8 *trans*-Anethole **CAS No. 104-46-1** Anethole (unspecified isomer)	A, Calc	A, Calc	A, Calc	Calc	A, Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 4180-23-8 *trans*-Anethole **CAS No. 104-46-1** Anethole (unspecified isomer)	Calc	NA	A	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 4180-23-8 *trans*-Anethole **CAS No. 104-46-1** Anethole (unspecified isomer)	A, Calc	A, Calc	A, Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 4180-23-8 *trans*-Anethole **CAS No. 104-46-1** Anethole (unspecified isomer)	A	A	A	A	A	A

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

4 REFERENCES FOR TEST PLAN AND ROBUST SUMMARIES

Al-Harbi M.M., Qureshi, S., Raza, M., Ahmed, M.M., Giangreco, A.B., and Shah, A.H. (1995) Influence of anethole treatment on the tumour induced by Ehrlick ascites carcinoma in paw of Swiss albino mice. *Eur J Cancer Prevent.,* **4**:307-318.

Argus Research Laboratories, Inc. (1992) Reproductive and developmental toxicity screening test of (anethole) administered orally *via* gavage to Crl:CDBR VAF/Plus female rats. Final Report. September 14, 1992.

AOPWIN EPI Suite (2000) U S Environmental Protection Agency.

Bauer K. and D. Garbe (1985) *Common Flavor and Fragrance Materials* Verlagsgesellschaft mbH, D-6940, Weinheim, Federal Republic of Germany.

Bauer K. and Molleken R. (1974) E/AS 2 418 974 (1974) Haarmann & Reimer GmbH.

Boissier J.-R., Simon, P., and Le Bourhis, B. (1967) Action psychotrope expérimentale des anéthole isomères cis et trans. *Therapie,* **22**:309-323.

Borriston Laboratories, Inc. (1982) Evaluation of immunosuppression in B6C3F1 mice with [anethole]. Final Report dated July 28, 1982. Borriston Laboratories, Inc., Temple Hills, MD.

Borriston Laboratories, Inc. (1984) 14-Day single dose subacute toxicity study in the rat with [*trans*-anethole]. Borriston Laboratories, Inc., Temple Hills, MD. Unpublished Final Report.

Bounds S.V. (1994) Dissertation Thesis. Disposition *trans*-anethole in rats and mice. Unpublished Report to FEMA.

Bounds S.V. and Caldwell, J. (1992) Influence of pre-feeding upon the disposition of [(^{14})C]-*trans*-anethole in the Sprague-Dawley CD rat. *ISSX International Meeting* **2**:159.

Bounds S.V. and Caldwell, J. (1996) Pathways of metabolism of [1'-^{14}C] *trans*-anethole in the rat and mouse. *Drug Metabolism and Disposition* **24**:717-724.

Broderius S., Hammermeister, D., Russom, C. (1990) Toxicity of eight terpenes to fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), and algae (S*elanastrum capriucornutum*). US EPA Environmental Research Laboratory/AScI Corporation. Unpublished.

Caldwell J. (1991) *trans*-Anethole—Another nongenotoxic rodent carcinogen. Presented at the Toxicology Forum 15-19 July, pp. 616-639. Aspen, Colorado.

Caldwell J. and Sutton, J.D. (1988) Influence of dose size on the disposition of *trans*-(methoxy-^{14}C) anethole in human volunteers. *Fd Chem Toxico.,l* **26**:87-91.

Caldwell J., Marshall, A.D., Reed, P., and Bounds, S.V.J. (1991) Metabolic and mechanistic studies to underpin the safety assessment of *trans*-anethole. Unpublished interim report of the Anethole Task Force of FEMA.

Caldwell J., Chan, V.S.W., Marshall, A.D., Hasheminejad, G., and Bounds, S.V.J. (1992) 1'-Hydroxylation is the only metabolic pathway of simple alkenylbenzenes involved in their genotoxicity. *Toxicologist,* **12**:56.

Caujolle F. and Meynier, D. (1958) Toxicité de l'estragole et des anétholes (*cis et trans*). *Séance du 3 Mars*: 1465-1468.

CIVO-TNO (2000) *Volatile Components in Food-Qualitative and Quantitative Data.* Supplement 5 to the 6th Edition. Edited by H. Maarse, C.A. Visscher, L.C. Willemsens, L.M. Nijssen, and M.H. Boelens. TNO Nutrition and Food Research. Zeist, The Netherlands.

CRC Handbook of Chemistry and Physics (1995) 75th Edition. Lide, D.R. editor. CRC The Chemical Rubber Co. Press Inc., Boca Raton, FL.

Daubert T.E., and Danner, R.P. (1989) Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Taylor and Francis, Washington, DC.

Derfer and Traynor (1992) The Chemistry of Turpentine in "Naval Stores", Editors D. Zinkel and J. Russell.Pulp Chemicals Association. New York, New York.

ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Fragrance Materials Association (FMA) Unpublished report.

Fritsch P., De Saint Blanquat, G., and Derache, R. (1975) Gastrointestinal absorption in the rat of anisole, *trans*-anethole, butylhydroxyanisole and safrole. *Fd Cosmet Toxicol* **13**:359-363.

Gorelick N.J. (1995) Genotoxicity of *trans*-anethole *in vitro*. *Mutat Res* **326**:199-209.

Guenther, A., Hewitt, C., Erickson, D., Fall, R., Geron, C., Graedel, T., Harley, P., Klinger, L., and M. Lerdau (1995) A global model of natural volatile organic compound emissions. *J. Geophys. Res, [Atmos.],* **100, D5:** 8873-8892.

Guenther, A., Geron, C., Pierce, T., Lamb, B., Harley, P. and R. Fall (2000) Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America. *Atmos. Environ.*, **34**: 2205-2230.

Hagan E.C., Hansen W.H., Fitzhugh O.G., Jenner P.M., Jones W.I., Taylor J.M., Long E.L., Nelson A.A., and Brouwer J.B. (1967) Food flavorings and compounds of related structure. II. Subacute and chronic Toxicity. *Fd Cosmet Toxicol* **5**:141-157.

Hall R.L. and Oser, B.L. (1965) Recent progress in the consideration of flavoring ingredients under the food additives amendment. III. GRAS substances. *Food Technology* **19**:151-197.

Heck J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B. and Curren, R.D. (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *Toxicologist* **9**(1) 1989.

Howes A.J., Chan, V.S.W., and Caldwell, J. (1990) Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. *Fd Chem Toxic,* **28**(8):537-542.

Hsia M.T.S., Adamovics, J.A., and Kreamer, B.L. (1979) Microbial mutagenicity studies of insect growth regulators and other potential insecticidal compounds in *Salmonella typhimurium*. *Chemosphere,* **8**:521-529.

IIT Research Institute (1995a) Immunomodulatory screening test of [*trans*-anethole] administered orally *via* gavage to B6C3F1 mice. Unpublished report.

IIT Research Institute (1995b) Immunomodulatory screening test of [*trans*-anethole] administered orally *via* gavage to B6C3F1 mice. Unpublished report.

Interactive Analysis LogP and LogW Predictor: Database contributed by Syracuse Research Corporation, SciVision, Albany Molecular Research, Inc., eduSoft LC, Cambridge Soft. www.logp.com.

Jenner P.M., Hagen, E.C., Taylor, J.M., Cook, E.L., and Fitzhugh, O.G. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. *Fd Cosmet Toxicol* **2**:327-343.

Klimisch H. J., Andreae, M., and U. Tillman (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Journal of Regulatory Toxicology and Pharmacology*, **25**, 1-5.

KOWWIN EPI Suite (2000) U S Environmental Protection Agency, (Hansch C. *et al*., 1995).

Lawrence B.M. (1985) A review of the world production of essential oils (1984) *Perf. and Flav*., **10(5),** 1-16.

Le Bourhis B. (1968) Preliminary research in the metabolism of *trans*-anethole. *Annales Biologique Clinique* **26**:711-715.

Le Bourhis B. (1970) Identification of some metabolites of *trans*-anethole in man, rabbits and rats. *Annals pharmacie francais,* **28**:355-361.

Le Bourhis B. (1973a) Biological properties of *trans*-anethole. An attempt to determine the acceptable daily dose. *Parfums Cosmetiques Savons France* **3**:8-9; 450-456.

Le Bourhis B. (1973b) 4-Generation reproduction study in rats given *trans*-anethole in the diet. Unpublished report by Sophie Holm. Laboratoire de Physiologie, Institut de Recherches appliquées aux Boissons, Montreuil, **93**, France.

Lucas C.D., Putman J.M., and Hallagan J.B. (1999) Flavor and Extract Manufacturers Association (FEMA) of the United States 1995 poundage and technical effects update survey. Washington, DC. Unpublished report.

Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626.

Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Marshall A.D., Howes, A.J. and Caldwell, J. (1989) Cytotoxicity and genotoxicity of the food flavour anethole in cultured rat hepatocytes. *Hum Toxicol,* **8**(5):404.

Marshall, A.D., and Caldwell, J. (1996) Lack of influence of modulators of epoxide metabolism on the genotoxicity of *trans*-anethole in freshly isolated rat hepatocytes assessed with the unscheduled DNA synthesis assay. *Fd Chem Toxicol,* **34**:337-345.

McKibben R. (1979) A History of SCM Organic Chemicals. Millennium Chemicals. Unpublished report.

Merck Index (1997) Merck & Co., 13th edition, Inc. Whitehouse Station, NJ.

Millennium Chemicals (2000) Composition of isolated intermediates. Unpublished report.

Miller E.C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A., and Miller, J.A. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Res* **43**:1124-1134.

Minnema D.J. (1997a) 28-Day range-finding dietary toxicity study of *trans*-anethole in rats. CHV 2595-102. Corning Hazleton Inc., Vienna, VA.

Minnema D.J. (1997b) 28-Day range-finding dietary toxicity study of *trans*-anethole in mice. CHV 2595-101. Corning Hazleton Inc., Vienna, VA.

Minnema D.J. (1997c) 90-Day subchronic dietary toxicity study of trans-anethole in mice. CHV2595-103. Corning Hazletone Inc. Vienna, VA.

Minnema D.J. (1997d) 90-Day subchronic dietary toxicity study of trans-anethole in rats. CHV2595-104. Corning Hazletone Inc. Vienna, VA.

Mortelmans K., Haworth, S., Lawlor, R., Speck, W., Tainer, B. and Zeiger, E. (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutagen*. **8**(Suppl.7):1-119.

MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

Muller L., Kasper, P., Muller-Tegethoff, K., and Petr, T. (1994) The genotoxic potential *in vitro* and *in vivo* of the allyl benzene etheric oils estragole, basil oil and *trans*-anethole. *Mutat Res* **325**:129-136.

National Resources Institute (1995) Gum naval stores: turpentine and rosin from pine resin. In Non-wood forest products 2, publisher: Food and Agriculture Organization of the United Nations, Rome.

Nestmann E.R., and Lee, E.G.-H. (1983) Mutagenicity of constituents of pulp and paper mill effluent in growing cells of *Saccharomyces cerevisiae*. *Mutat Res*, **119**:273-280.

Nestmann E.R., Lee, E.G.-H. , Matula, T.I., Douglas, G.R., and Mueller, J.C. (1980) Mutagenicity of constituents identified in pulp and paper mill effluents using the *Salmonella*/mammalian-microsome assay. *Mutat Res*, **79**:203-212.

Newberne P.M. (1997) Histopathological evaluation of livers of mice and rats. Unpublished report to the *trans*-anethole Committee of the Flavor and Extract Manufacturers' Association. June 2, 1997.

Newberne P.M., Carlton, W.W., and Brown, W.R. (1989) Histopathological evaluation of proliferative liver lesions in rats fed *trans*-anethole in chronic studies. *Fd Chem Toxicol* **27**(1):21-26.

Newberne R., Smith, R.L., Doull, J., Goodman, J.I., Munro, I.C., Portoghese, P.S., Wagner, B.M., Weil, C.S., Woods, L.A., Adams, T.B., Lucas, C.D., and Ford, R.A. (1999) The FEMA GRAS assessment of *trans*-anethole used as a flavoring substance. *Fd Chem Toxicol* **37**:789-811.

Phillips D.H., Reddy, M.V., and Randerath, K. (1984) ^{32}P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-

occurring alkenylbenzenes. II. Newborn male B6C3F1 mice. *Carcinogenesis* **5(12):**1623-1628.

Quest International, Inc. (1994) The ultimate and readily biodegradation of anethole. Unpublished report.

Randerath K., Haglund, R.E., Phillips, D.H., and Reddy, M.V. (1984) ^{32}P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice. *Carcinogenesis* **5(12**):1613-1622.

Reed P.M. (1994) Hepatocellular changes induced by *trans*-anethole in rodents. A Thesis submitted for the Degree of Doctor of Philosophy in the University of London. Dated March 1994.

Reed P.M. and Caldwell, J. (1992a) Induction of cytochrome P450 and related enzyme activities following dietary administration of *trans*-anethole to Sprague-Dawley CD rats. *Hum Exp Toxicol,* **11**(6):580-581.

Reed P.M. and Caldwell, J. (1992b) Effects of dietary administration of *trans*-anethole on the liver of the Sprague-Dawley CD rat. *Toxicol Lett Suppl*: 283. Presented at the 6th International Congress of Toxicology, Rome, 1992.

Reed P.M. and Caldwell, J. (1993) The effects of dietary administration of the food flavour *trans*-anethole on mouse liver. *Hum Exp Toxicol,* **12(6):**565.

Sangster S.A., Caldwell, J., Smith, R.L., and Farmer, P.B. (1984a) Metabolism of anethole. I. Pathways of metabolism in the rat and mouse. *Fd Chem Toxicol* **22**:695-706.

Sangster S.A., Caldwell, J., and Smith, R.L. (1984b) Metabolism of anethole. II. Influence of dose size on the route of metabolism of *trans*-anethole in the rat and mouse. *Fd Chem Toxicol,* **22**:707-713.

Sangster S.A., Caldwell, J., Hutt, A.J., Anthony, A., and Smith, R.L. (1987) The metabolic disposition of (methoxy-^{14}C)-labeled *trans*-anethole, estragole and *p*-propylanisole in human volunteers. *Xenobiotica* **17**:1223-1232.

Sekizawa J. and Shibamoto, T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. *Mutat Res* **101**:127-140.

Solheim E. and Scheline, R.R. (1973) Metabolism of alenebenzene derivatives in the rat I. *p*-Methoxyallylbenzene(estragole) and *p*-methoxypropenylbenzene (anethole). *Xenobiotica* **3**:493-510.

Solheim E. and Scheline, R.R. (1976) Metabolism of alkenebenzene derivatives in the rat. II. Eugenol and isoeugenol methyl ethers. *Xenobiotica* **6**:137-150.

Svadkowskaya G. E., Kheifits, L.A., Platova, A.I., and K. Denisenlova (1970) All-Union Scientific Research Institute of Synthetic and Natural Perfumes. SU 261 380.

Swanson A.B., Chambliss, D.D., Blomquist, J.C., Miller, E.C., Miller, J.A. (1979) The mutagenicities of safrole, estragole, eugenol, *trans*-anethole, and some of their known or possible metabolites for *Salmonella typhimurium* mutants. *Mutat Res,* **60**:143-153.

Taylor J.M., Jenner, P.M., and Jones, W.I. (1964) A comparison of the toxicity of some allyl, propenyl, and propyl compounds in the rat. *Toxicol Appl Pharmacol,* **6**:378-387.

To L.P., Hunt, T.P., Andersen, M.E. (1982) Mutagenicity of *trans*-anethole, estragole, eugenol, and safrole in the Ames Salmonella typhimurium assay. *Bull Environ Contam Toxicol,* **28**:647-654.

Truhaut R., Le Bouhis, B., Attia, M., Glomot, R., Newman, J., and Caldwell, J. (1989) Chronic toxicity/carcinogenicity study of *trans*-anethole in rats. *Fd Chem Toxicol,* **27(1):**11-20.

Wenk M.L. (1994) Induction of hepatic microsomal enzymes in rats by [anethole]. Unpublished reported dated June 13, 1994. Microbiological Associates, Inc., Bethesda, MD.

WSKOW EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski S.H., and Dannenfelser, R.M., 1992)

WSKOW EPI Suite (2000b) U S Environmental Protection Agency.

AR201-14069B

The Flavor And Fragrance High Production Volume Consortia

The Terpene Consortium

Robust Summaries for Anethole (isomer unspecified) and *trans*-Anethole

Anethole (isomer unspecified) **CAS No. 104-46-1**

***trans*-Anethole** **CAS No. 4180-23-8**

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
The Flavor and Fragrance High Production Volume Chemical Consortia
1620 I Street, NW, Suite 925
Washington, DC 20006
Phone: 202-331-2325
Fax: 202-463-8998

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The Flavor and Fragrance High Production Volume Consortia

Robust Summaries for Anethole (isomer unspecified) and *trans*-Anethole

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 Melting Point

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Measured
GLP	Ambiguous
Melting Point	21.3 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	CRC Handbook of Chemistry and Physics (1995) 75th ed., D. R. Lide ed., The Chemical Rubber Co. Press Inc., FL.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Measured
GLP	Ambiguous
Melting Point	21.4 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Merck Index (1997) Merck & Co., Inc. Whitehouse Station, NJ.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated/Mean or weighted
Melting Point	-0.69 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

1.2 Boiling Point

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	234 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	CRC Handbook of Chemistry and Physics (1995) 75th edition, D. R. Lide editor, The Chemical Rubber Co. Press Inc., Boca Raton, FL.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated
GLP	Ambiguous
Boiling Point	236 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Fragrance Materials Association (FMA) Unpublished report.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated\adapted Stein and Brown method
Boiling Point	217.31 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

1.3 Vapor Pressure

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Measured
GLP	Ambiguous
Vapor Pressure	0.041 mm Hg (5.45 Pa)
Temperature	21 °C (294 K)
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Daubert T.E. and Danner, R.P. (1989) Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Taylor and Francis, Washington, DC.

Compilation. Taylor and Francis, Washington, DC.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated/Antoine and Grain methods
Vapor Pressure	0.0634 mm Hg (8.45 Pa)
Temperature	25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated
Vapor Pressure	0.05 mm Hg (6.67 Pa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Unpublished report.

1.4 n-Octanol/Water Partition Coefficients

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated
Log Pow	3.11
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Interactive Analysis LogP and LogW Predictor: Database contributed by Syracuse Research Corporation, SciVision, Albany Molecular Research, Inc., eduSoft LC, Cambridge Soft.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated
Log Pow	3.39
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) U S Environmental Protection Agency, (Hansch C. *et al*., 1995).

1.5 Water Solubility

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/Guideline	Measured
GLP	No
Value (mg/L) at Temperature	111 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: peer reviewed reference
References	WSKOW EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski S.H., and Dannenfelser, R.M., 1992)

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/Guideline	Calculated
Value (mg/L) at Temperature	139.8 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOW EPI Suite (2000b) U S Environmental Protection Agency.

Substance Name	*trans*-Anethole

CAS No.	4180-23-8
Method/Guideline	Calculated
GLP	No
Value (mg/L) at Temperature	285.384 mg/L
Remarks for Results	No temperature given.
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Interactive Analysis LogP and LogW Predictor: Database contributed by Syracuse Research Corporation, SciVision, Albany Molecular Research, Inc., eduSoft LC, Cambridge Soft.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 Photodegradation

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Calculated
Test Type	AOPWIN
Halflife t1/2	2.015 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) U S Environmental Protection Agency.

2.2 Biodegradation

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method	OECD Guideline 301B
Year	1994
Innoculum	10% by volume of secondary effluent from an unacclimatized activated sludge
Degradation % After Time	91.0% (90.7-91.2%)
Remarks	The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 20-24 °C. The mean percentage biodegradation was calculated from 4 vessels on day 28.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Quest International, Inc. (1994) The ultimate and readily biodegradation of anethole. Unpublished report.

2.3 Fugacity

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Air
Estimated Distribution and Media Concentration	0.53%
Model Data and Results	Half-life = 1.82 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626. Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Water

Model Data and Results	Half-life = 360 hours
Estimated Distribution and Media Concentration	29.8%
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626. Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Soil
Model Data and Results	Half-life = 360 hours
Estimated Distribution and Media Concentration	69.1 %
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626. Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Sediment
Model Data and Results	Half-life = 1440 hours
Estimated Distribution and Media Concentration	0.60 %
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626. Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

3 ECOTOXICITY

3.1 Acute Toxicity to Fish

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 99%
Method/guideline	96-hour LC50 continuous flow (ASTM, 1989)
Test Type	Experimental
GLP	Ambiguous
Year	1989
Species/Strain/Supplier	Minnows/Fathead
Exposure Period	96 hour
Analytical monitoring	GC Analysis
Remarks for Test Conditions	Temperature = 24.8 °C, dissolved oxygen = 6.4 mg/L, hardness = 39.4 mg/L CaCO3, alkalinity 30.6 mg/L CaCO3, tank volume = 1 L, pH = 7.6 Fish sizes: mean length = 16.7 mm; mean weight = 0.07 mm; loading 1.4 g/L; age = 30 days Stock solutions (49 mg/L) were prepared daily and supplied to the proportional diluter.
Observations of Precipitation	None
Endpoint value	LC50 = 7.690 mg/L; EC50 = 4.810 mg/L
Nominal concentrations as mg/L	0, 4,60, 7.08, 10.9, 16,8, and 25.8 mg/L
Measured concentrations as mg/L	Corrected average: Less than 0.06, 2.73, 3.96, 5.85, 10.1, and 17.2
Remarks fields for results	Confidence limits could not be reliably calculated. Test tanks were not sampled at 96 hours. Volatility caused actual concentrations to be less than nominal.
Unit	mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.

Reference	Broderius S., Hammermeister, D., Russom, C. (1990) Toxicity of eight terpenes to fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), and algae (*Selanastrum capriucornutum).* US EPA Environmental Research Laboratory/AScI Corporation. Unpublished. ASTM. 1989. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E729. In: Vol. 11.04 of 1989 Annual Book of ASTM Standards. American Society of Testing and Materials, Philadelphia, PA. pp. 336-355.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Based on: log KOW = 3.39, MP = 21.35 °C, water solubility = 111 mg/L
Endpoint value	96 hour LC50 = 5.423 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	14 days
Remarks for Test Conditions	Based on: log KOW = 3.39, MP = 21.35 °C, water solubility = 111 mg/L
Endpoint value	14-day LC50 = 12.251 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.

Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish (SW)
Exposure Period	96 hour
Remarks for Test Conditions	Based on: log KOW = 3.39, MP = 21.35 °C, water solubility = 111 mg/L
Endpoint value	96-hour LC50 = 2.433 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 99%
Method/guideline	48-hour LC50 continuous flow (ASTM, 1989)
Test Type	Experimental
GLP	Ambiguous
Year	1989
Species/Strain/Supplier	*Daphnia magna*
Analytical procedures	GLC Analysis
Test Details	48 hours
Remarks for Test Conditions	Temperature =1 9.7 °C, dissolved oxygen = 7.8 mg/L, hardness = 45.5 mg/L CaCO3, alkalinity 36.8 mg/L CaCO3, tank volume = 0.20 L, pH = 8.0 *Daphnid* age less than 24 hours

	Stock solution =1 5.2 mg/L
Nominal concentrations as mg/L	0, 3.04, 6.08, 9.12, 12.2, and 15.2 mg/L
Measured concentrations as mg/L	Corrected average = Less than 0.06, 2.84, 5.42, 7.13, 10.9, and 14.5 mg/L
Unit	mg/L
EC50, EL50, LC0, at 24,48 hours	48-hour LC50 = 6.82 mg/L (CL: 6.30-7.39); 48-hour EC50 = 4.25 mg/L (CL: 3.89-4.65)
Appropriate statistical evaluations?	Yes
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Data Reliability Remarks	Code 1. Comparable to guideline study.
Reference	Broderius S., Hammermeister, D., Russom, C. (1990) Toxicity of eight terpenes to fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), and algae (*Selanastrum capriucornutum)*. US EPA Environmental Research Laboratory/AScI Corporation. Unpublished. ASTM. 1989. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E729. In: Vol. 11.04 of 1989 Annual Book of ASTM Standards. American Society of Testing and Materials, Philadelphia, PA. pp. 336-355.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	ECOSAR
Test Type	Calculated
GLP	No
Species/Strain/Supplier	*Daphnia magna*
Test Details	48 hours
Remarks for Test Conditions	Based on: log KOW = 3.39, MP = 21.35 °C, water solubility = 111 mg/L
EC50, EL50, LC0, at 24,48 hours	48-hour LC50 = 6.397 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	ECOSAR
Test Type	Calculated
GLP	No
Species/Strain/Supplier	*Daphnia magna*
Test Details	16 days
Remarks for Test Conditions	Based on: log KOW = 3.39, MP = 21.35 °C, water solubility = 111 mg/L
EC50, EL50, LC0, at 24,48 hours	16-day EC50 = 0.603 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	ECOSAR
Test Type	Calculated
GLP	No
Species/Strain/Supplier	Mysid shrimp
Test Details	96 hours
Remarks for Test Conditions	Based on: log KOW = 3.39, MP = 21.35 °C, water solubility = 111 mg/L
EC50, EL50, LC0, at 24,48 hours	96-hour LC50 = 0.58 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

3.3 Acute Toxicity to Aquatic Plants

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Static 96-hour toxicity test (ASTM, 1988)
Test Type	Experimental
GLP	Ambiguous
Year	1989
Species/Strain/Supplier	Green algae
Exposure Period	72 to 96 hours
Remarks for Test Conditions	Because of volatility issues, 75 mL of test solution were placed in 125 mL flasks to minimize headspace. Five concentrations of stock were tested: 100, 50, 25, 12.5, and 0% in replicates of 4 and shaken continuously. Test cell concentrations were about 1x10E4 cell/mL. IC50 was calculated using a linear interpolation program (Marcus and Holtzman, 1988; Norberg-King, 1988)
Endpoint basis	IC50
Endpoint Value	96-hour IC50 = 9.571 mg/L (CI:7.434-13.274)
Analytical monitoring	GC analysis
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Broderius S., Hammermeister, D., Russom, C. (1990) Toxicity of eight terpenes to fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), and algae (*Selanastrum capriucornutum).* US EPA Environmental Research Laboratory/AScI Corporation. Unpublished report.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	ECOSAR
Test Type	Calculated
GLP	No
Species/Strain/Supplier	Green algae

Exposure Period	96 hour
Remarks for Test Conditions	Based on: log KOW = 3.39, MP = 21.35 °C, water solubility = 111 mg/L
Endpoint basis	EC50
Endpoint Value	EC50 = 4.332 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

4 HUMAN HEALTH TOXICITY

4.1 Acute Toxicity

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Litchfield and Wilcoxon, 1949
Test Type	Acute oral LD50
GLP	No
Year	1964
Species/strain	Rat/Osborne-Mendel
Sex	Male and Female
# of animals per sex per dose	5
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Five male and five female young adult Osborne-Mendel rats were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Value LD50 or LC50 with confidence limits	2090 mg/kg bw (95% C.L. 1420-3070)
Remarks for Results	Slope function: 1.8 (95% C.L. 1.3-2.4). Toxic signs were depression at low doses and coma at high doses. Time of death was between 4 hours and 4 days.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Jenner P.M., Hagen, E.C., Taylor, J.M., Cook, E.L., and Fitzhugh, O.G. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. Fd Cosmet Toxicol 2:327-343.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Test Type	Macroscopic liver lesions

GLP	No
Year	1964
Species/strain	Rat
Sex	Male and Female
Number of animals per sex per dose	3
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Groups of 3 male and 3 female rats were gavaged with 695 mg anethole/kg bw/day for 4 days. Rats were killed on the 5th day. Livers were removed and examined for gross lesions. Lesions were rated and individual liver ratings were averaged to provide an overall rating for anethole.
Number of deaths at each dose level	At 464 mg/kg bw: 0 At 681 mg/kg bw: 3 At 1,000, 1,470, and 2,150 mg/kg bw: all
Remarks for Test Conditions	At 695 mg/kg bw, 1/6 rats died.
Remarks for Results	Mild liver lesions were reported consisting of slight discoloration, mottling, and blunting of the lobe edges. The authors noted that many of the rats lost weight and were in poor condition.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Taylor J.M., Jenner, P.M., and Jones, W.I. (1964) A comparison of the toxicity of some allyl, propenyl, and propyl compounds in the rat. Toxicol Appl Pharmacol., 6, 378-387.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/Guideline	Litchfield and Wilcoxon, 1949
Test Type	Acute oral LD50
GLP	No
Year	1964
Species/strain	Mouse
Sex	Not reported
Number of animals per sex per dose	Not stated
Route of Administration	Oral-Gavage

Remarks for Test Conditions	Groups of mice were treated on full stomachs. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Value LD50 or LC50 with confidence limits	3050 mg/kg bw (95% C.L. 2330-4000)
Remarks for Results	Slope function: 1.6 (95% C.L. 1.2-2.1). Toxic signs were depression and coma within 15 min. Time of death was between 2 and 4 hours.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Jenner P.M., Hagen, E.C., Taylor, J.M., Cook, E.L., and Fitzhugh, O.G. (1964). Food flavourings and compounds of related structure. I. Acute oral toxicity. Fd Cosmet Toxicol 2:327-343.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/Guideline	Litchfield and Wilcoxon, 1949
Test Type	Acute oral LD50
GLP	No
Year	1964
Species/strain	Guinea pig
Sex	Male and Female
Number of animals per sex per dose	Not stated
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Groups of guinea pigs consisting of both males and females were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Value LD50 or LC50 with confidence limits	2160 mg/kg bw (95% C.L. 1920-2450)
Remarks for Results	Slope function: 1.3 (95% C.L. 1.2-1.5). Toxic signs were depression. Time of death was between 1 and 7 days.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Jenner P.M., Hagen, E.C., Taylor, J.M., Cook, E.L., and Fitzhugh, O.G. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. Fd Cosmet Toxicol 2:327-343.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/Guideline	LD50 method of Miller and Tainter, 1944
Test Type	Acute oral LD50
GLP	No
Year	1967
Species/strain	Rat
Sex	Male
Number of animals per sex per dose	12
Route of Administration	Oral
Value LD50 or LC50 with confidence limits	3,200+/-300 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Boissier J.-R., Simon, P., and Le Bourhis, B. (1967) Action psychotrope experimentale des anethole isomeres cis et *trans*. Therapie 22:309-323.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/Guideline	LD50 method of Miller and Tainter, 1944
Test Type	Acute LD50
GLP	No
Year	1967
Species/strain	Mouse
Sex	Male
Number of animals per sex per dose	12
Route of Administration	Intraperitoneal Oral
Value LD50 or LC50 with confidence limits	650+/-36 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.

Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Boissier J.-R., Simon, P., and Le Bourhis, B. (1967) Action psychotrope experimentale des anethole isomeres cis et *trans*. Therapie 22:309-323.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/Guideline	LD50 method of Miller and Tainter, 1944
Test Type	Acute oral LD50
GLP	No
Year	1967
Species/strain	Mouse
Sex	Male
Number of animals per sex per dose	12
Route of Administration	Oral
Value LD50 or LC50 with confidence limits	5,000+/-900 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Boissier J.-R., Simon, P., and Le Bourhis, B. (1967) Action psychotrope experimentale des anethole isomeres cis et *trans*. Therapie 22:309-323.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Test Type	Acute LD50
GLP	No
Year	1958
Species/strain	Mouse/Swiss
Sex	Not reported
Route of Administration	Intraperitoneal
Remarks for Test Conditions	Mice were administered 500, 700, 1000, 1500, 2000, 3000, 5000 or 10000 mg *trans*-anethole/kg bw and observed for 20 hours.

Value LD50 or LC50 with confidence limits	1410 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Caujolle F. and Meynier, D. (1958) Toxicite de l'estragole et des anetholes (cis et *trans*). Séance du 3 Mars: 1465-1468.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/Guideline	Litchfield and Wilcoxon, 1949
Test Type	Acute LD50
GLP	No
Year	1984
Species/strain	Rat/Sprague-Dawley
Sex	Male and Female
Number of animals per sex per dose	5
Vehicle	1% methylcellulose
Route of Administration	Intraperitoneal
Remarks for Test Conditions	Rats were given a single intraperitoneal of 464, 681, 1,000, 1,470, or 2,150 mg *trans*-anethole/kg bw. Rats were observed for 15 days and gross necropsies were performed.
Value LD50 or LC50 with confidence limits	Combined sexes: 718 (CL: 566-912) mg/kg bw Males: 738 (CL: 525-1,038) mg/kg bw Females: 703 (CL: 473-1,044) mg/kg bw
Number of deaths at each dose level	At 464 mg/kg bw: 0 At 681 mg/kg bw: 3 At 1,000, 1,470, and 2,150 mg/kg bw: all
Remarks for Results	At the 2 highest doses, rats died within 3 hours of treatment. At 1,000 mg/kg bw, rats died by day 2. White areas on the surface of the spleen and/or enlarged spleen noted in rats of the 2 low-dose groups. Distended stomachs noted at 1,000 mg/kg bw and mottled livers reported in rats from the 2 highest dose groups.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borriston Laboratories, Inc. (1984) 14-Day single dose subacute toxicity study in the rat with [*trans*-anethole]. Unpublished Final Report.

Unpublished Final Report.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/Guideline	LD50 method of Miller and Tainter, 1944
Test Type	Acute LD50
GLP	No
Year	1967
Species/strain	Rat
Sex	Male
Number of animals per sex per dose	12
Route of Administration	Intraperitoneal
Value LD50 or LC50 with confidence limits	900+/-45 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Boissier J.-R., Simon, P., and Le Bourhis, B. (1967) Action psychotrope experimentale des anethole isomeres cis et *trans*. Therapie 22:309-323.

4.2 Genetic Toxicity

4.2.1 *In vitro* Genotoxicity

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Remarks for Substance	Purity 98.9%
Method/guideline	Ames assay
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1982

Species/Strain	*Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, TA1538
Metabolic Activation	S9 prepared from PCB-treated male Sprague-Dawley rat liver
Doses/Concentration	60, 120, 300, 600 ug/plate
Remarks for Test Conditions	Assays without S9 were conducted by the plate-incorporation method and assays with S9 used the pre-incubation method. Anethole was dissolved in DMSO, which was also used as the vehicle control. Revertants/plate were an average of 3-5 replications.
Results	A significant increase in revertants in the presence of S9 was reported in strain TA100 as a linear dose response up to 120 ug/plate. Due to the bactericidal action of anethole, the number of revertants did not reach twice that of controls. The number of induced revertants was normalized for the number of viable cells by method of Green and Muriel (1976) and resulted in induced mutation frequencies of 1.58E-7, 3.74E-7, 6.59E-7, 1.01E-6 and 1.31E-6 at concentrations of 30, 60, 90, 120 and 150 ug/plate. Anethole did not induce a significant increase in revertants with or without S9 in the other strains.
Cytotoxic concentration	Not given
Genotoxic Effects	Induction of revertants in strain TA100 in the presence of S9. No induction of revertants in strains TA1535, TA98, TA1537, or TA1538
Remarks for Results	Although not clearly stated in the article, it appears that the study with strain TA100 in the presence of S9 was repeated at 30, 60, 90, 120, and 150 ug/plate to confirm the original findings.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Sekizawa J. and Shibamoto, T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutat Res 101:127-140.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Ames assay
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1979

Species/Strain	*Salmonella typhimurium* strain TA98, TA100, TA1535, TA1537, and TA1538
Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced Sprague-Dawley rat
Doses/Concentration	2, 20, or 200 ug/plate
Remarks for Test Conditions	Solvent control used was DMSO. Positive controls used were benzo(a)pyrene (BP), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and 2-aminofluorene (AF).
Results	The following values give the number of His+ revertants/plate for the DMSO control, and at 2, 20, and 200 ug/plate, respectively. TA98: 44, 79, 70, and 65 TA100: 97, 177, 159, and 196 TA1535: 21, 26, 33, and 27 TA1537: 18, 40, 47, and 40 TA1538: 35, 53, 59, and 58
Cytotoxic concentration	Not given
Genotoxic Effects	None
Remarks for Results	Authors noted that anethole may show weak activity in TA100, but there was no clear dose-response.
Conclusion Remarks	Anethole was not mutagenic in this assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Hsia M.T.S., Adamovics, J.A., and Kreamer, B.L. (1979) Microbial mutagenicity studies of insect growth regulators and other potential insecticidal compounds in Salmonella typhimurium. Chemosphere 8:521-529.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Ames assay
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1989
Species/Strain	*Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100

Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced male Sprague-Dawley rat
Doses/Concentration	25,000 ug/plate
Remarks for Test Conditions	Following 2 days of incubation at 37 C, revertant colonies were counted electronically.
Results	No genotoxic effects were observed.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	*trans*-Anethole was inactive in the Ames assay using *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 with or without S9 activation.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Heck J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B. and Curren, R.D. (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1) 1989.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Mouse lymphoma assay (Clive *et al*. 1979)
Test Type	Forward mutation test
System of Testing	Mammalian
GLP	No
Year	1989
Species/Strain	L5178Y mouse lymphoma cell line
Metabolic Activation	Rat liver microsome fraction S9 and cofactors
Doses/Concentration	With S9: 62.5 nl/ml (highest inactive dose tested) Without S9: 7.8, 15.6-31.3 nl/ml
Remarks for Test Conditions	Cells were exposed to *trans*-anethole for 4 hours, washed, incubated for 48 hours and then cloned. After 10-14 days, colonies were automatically counted. The ratio of mutant to viable colonies cloned without selective medium was considered to be the mutant frequency.
Results	No increase in mutagenesis (as compared to the negative controls) except at 15.6-31.3 nl/ml (with S9) where a 2.9- to 4.6-fold increase was observed.
Cytotoxic concentration	Not given

Genotoxic Effects	Increased mutations with S9
Conclusion Remarks	Although *trans*-anethole produced an increase in mutagenic activity at concentrations ranging from 15.6-31.3 nl/ml (with S9), no change in mutagenic activity was reported at the other concentrations. Without further detail regarding the study design, it is difficult to interpret the significance of the positive finding.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Heck J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B. and Curren, R.D. (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1) 1989.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Ames assay
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1980
Species/Strain	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538
Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced rat
Doses/Concentration	Up to 0.2 mg/plate
Remarks for Test Conditions	DMSO used as vehicle control.
Results	No increase in His+ revertants at doses high enough to cause lethality
Cytotoxic concentration	Greater than 0.2 mg/plate
Genotoxic Effects	None
Conclusion Remarks	Anethole was not mutagenic in this assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Nestmann E.R., Lee, E.G.-H. , Matula, T.I., Douglas, G.R., and Mueller, J.C. (1980) Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian-microsome assay. Mutat Res 79:203-212.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Remarks for Substance	Purity 98.9%
Method/guideline	Ames (Green and Muriel, 1976)
Test Type	Reversion test
System of Testing	Bacterial
GLP	No
Year	1982
Species/Strain	*Escherichia coli* WP2 uvrA trp-
Metabolic Activation	S9 prepared from PCB-treated male Sprague-Dawley rat liver
Doses/Concentration	60, 120, 300, 600 ug/plate
Remarks for Test Conditions	Modification of the Ames assay: 0.1 umole of tryptophan used as a supplement in the soft agar instead of 0.1 umole histidine plus 0.1 mole biotin. Tryptophan revertant colonies were scored. Anethole was dissolved in DMSO, which was also used as the vehicle control. Revertants/plate were an average of 3-5 replications.
Results	For DMSO control, 60, 120, 300, or 600 ug/plate, the number of revertants/plate was 55, 52, 45, 54, or 45, respectively, without S9 and 59, 62, 55, 41, or 48, respectively, with S9.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	Anethole did not induce revertants in *E. coli*
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Sekizawa J. and Shibamoto, T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutat Res 101:127-140.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 99%
Method/guideline	Ames assay
Test Type	Ames reverse mutation

System of Testing	Bacterial
GLP	No
Year	1979
Species/Strain	*Salmonella typhimurium* strain TA1535, TA98, and TA100
Metabolic Activation	S13 fractions from the liver of Aroclor 1254-induced CD rats
Doses/Concentration	TA100: up to 20 umol/plate TA98: up to 30 umol/plate
Remarks for Test Conditions	When S13 was added, the agar was fortified with 0.5 mL of an NADPH-generating system.
Results	TA100: results were shown graphically. The number of revertants in the control was approximately 120/plate. Without S13, anethole did not induce an increase in the frequency of revertants (value appears to coincide with control at 20 umol/plate). With S13, anethole induced the number of revertants to over 600/plate at a concentration of 10 umol/plate. At 20 umol/plate the number of revertants dropped off to between 0 and 200/plate (likely due to cytotoxicity). TA98: no specific values were reported; however, it was stated that no mutagenic activity was observed at concentrations up to 30 umol/plate with or without metabolic activation with S13. TA1535: no results were reported with anethole
Cytotoxic concentration	Not given
Genotoxic Effects	Induction of revertants in strain TA100 in the presence of S13. No mutagenic effects in TA100 without S13 or in TA98 with or without S13.
Conclusion Remarks	*trans*-Anethole induced revertants in TA100 when metabolically activated, but showed no mutagenic activity in TA100 without S13 or in TA98.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Swanson A.B., Chambliss, D.D., Blomquist, J.C., Miller, E.C., Miller, J.A. (1979) The mutagenicities of safrole, estragole, eugenol, *trans*-anethole, and some of their known or possible metabolites for *Salmonella typhimurium* mutants. Mutat Res 60:143-153.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Ames assay (Haworth *et al*., 1983)
Test Type	Ames reverse mutation

System of Testing	Bacterial
GLP	Yes
Year	1986
Species/Strain	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537
Metabolic Activation	S9 fraction from the livers of Aroclor 1254-induced male Sprague-Dawley rats or Syrian hamsters
Doses/Concentration	0, 1.0, 3.3, 10.0, 33.0, 67.0, 100.0, or 200.0 ug/plate
Remarks for Test Conditions	Sodium azide (TA1535 and TA100), 4-nitro-o-phenylenediamine (TA98), and 9-aminoacridine (TA97 and TA1537) were used as positive controls for the specific Salmonella strains without S9. 2-Aminoanthracene was used with all strains incubated with S9. Solvent controls were also prepared concurrently. Preliminary tests were conducted to assess the cytotoxicity of the test compound and establish suitable concentrations for testing. At least 5 concentrations of the test chemicals (in triplicate) were incubated with or without S9 for 20 minutes after which plates were prepared and incubated at 37 deg C for 48 hours. A test chemical was considered "mutagenic" if there was a dose-related, reproducible increase in the number of revertants over background (not required to be 2-fold increase), "non mutagenic" if there was no increase, and "questionable" if there was no clear reproducible dose-related increase or "when the response was of insufficient magnitude to support a determination of mutagenicity".
Results	Anethole produced no increased incidence of mutation as compared to the vehicle controls, either with or without S9 mix.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	Anethole was inactive in the Ames assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with or without S9 activation.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Mortelmans K., Haworth, S., Lawlor, R., Speck, W., Tainer, B. and Zeiger, E. (1986) Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen. 8(Suppl.7):1-119.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Ames assay

Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1982
Species/Strain	*Salmonella typhimurium* strain TA1535, TA100, TA1537, TA1538, TA98
Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced rat
Doses/Concentration	0.05, 0.20, 1.0, 5.0, 15.0, or 50.0 ug/plate
Statistical Methods	Mann-Whitney U test (P less than 0.05)
Remarks for Test Conditions	*trans*-Anethole was dissolved in ethanol, which was the vehicle control. In a supplementary study, the cofactor 3'-phosphadenosine-5'-phophosulfate (PAPS) was added.
Results	No significant differences in the number of revertants for any of the strains tested with or without S9. When PAPS was added, it significantly affected the mutagenicity of *trans*-anethole in strain TA98. At concentrations of 1 ug/plate and higher, the number of revertants was significantly increased.
Cytotoxic concentration	1 mg/plate
Genotoxic Effects	None in the absence of cofactor PAPS. In strain 1535, when PAPS was added, *trans*-anethole induced revertants.
Conclusion Remarks	*trans*-Anethole did not show mutagenic activity in Salmonella strains TA1535, TA100, TA1537, TA1538, or TA98 when tested in the presence or absence of metabolic activation without cofactor PAPS. When PAPS was added, *trans*-anethole induced revertants in strain TA1535.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	To L.P., Hunt, T.P., Andersen, M.E. (1982) Mutagenicity of *trans*-anethole, estragole, eugenol, and safrole in the Ames *Salmonella typhimurium* assay. Bull Environ Contam Toxicol 28:647-654.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 98.9%
Method/guideline	Ames assay
Test Type	Ames reverse mutation

System of Testing	Bacterial
GLP	No
Year	1995
Species/Strain	*Salmonella typhimurium* strain TA100
Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced male Sprague-Dawley rat
Doses/Concentration	25, 35, 40, 45, 50, 75, 100, or 500 ug/plate
Statistical Methods	To be considered positive, *trans*-anethole must have caused a dose-related increase in the mean revertants/plate with a minimum of 2 increasing concentrations and the peak increase in mean revertants must have been greater than or equal to 2X the mean vehicle control value.
Remarks for Test Conditions	The assay was conducted using the preincubation method with an enhanced NADPH-generating system containing 7 mg microsomal protein/plate and 40% S9. Treatments were conducted in triplicate. Positive control was DMBA. Ethanol was the vehicle control.
Results	For the vehicle control, 25, 35, 40, 45, 50, 75, 100, or 500 ug/plate, and DMBA, the number of revertants/plate (mean of 3 replicates) was 149, 158, 167, 171, 136, 162, 148, 165, 150 and 449, respectively.
Cytotoxic concentration	500 ug/plate
Genotoxic Effects	None
Conclusion Remarks	*trans*-Anethole did not induce an increase in the number of revertants in this test system.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Gorelick N.J. (1995) Genotoxicity of *trans*-anethole in vitro. Mutat Res 326:199-209.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 98.9%
Method/guideline	Ames assay
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1995

Species/Strain	*Salmonella typhimurium* strain TA100
Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced male Sprague-Dawley rat
Doses/Concentration	100, 200, 300, 500, or 750 ug/plate
Statistical Methods	To be considered positive, *trans*-anethole must have caused a dose-related increase in the mean revertants/plate with a minimum of 2 increasing concentrations and the peak increase in mean revertants must have been greater than or equal to 2X the mean vehicle control value.
Remarks for Test Conditions	The assay was conducted using the plate incorporation method with an activation system containing 3'-phosphadenosine-5'-phophosulfate (PAPS) and 1.75 mg microsomal protein/plate (excluding DMBA+standard S9) with 10% S9. Treatments were conducted in triplicate. Positive control was DMBA. Ethanol was the vehicle control.
Results	For the vehicle control, 100, 200, 300, 500, or 750 ug/plate, DMBA+PAPS S9, and DMBA+standard S9, the number of revertants/plate (mean of 3 replicates) was 151, 196, 240, 222, 214, 223, 1,228, and 2,035, respectively.
Cytotoxic concentration	750 ug/plate
Genotoxic Effects	None
Conclusion Remarks	*trans*-Anethole did not induce an increase in the number of revertants in this test system.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Gorelick N.J. (1995) Genotoxicity of *trans*-anethole *in vitro*. Mutat Res 326:199-209.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	von Borstel *et al*. (1981)
Test Type	Reversion test
System of Testing	Yeast
GLP	No
Year	1983
Species/Strain	Saccharomyces cerevisiae D7 and XV185-14C
Metabolic Activation	None
Doses/Concentration	Not specified

Statistical Methods	Result considered positive if the number of mutants was higher than that of controls and if the calculated mutant frequency was at least double that of the solvent control
Remarks for Test Conditions	Treatments were conducted in triplicate.
Results	Negative response. No further details given.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	*trans*-Anethole did not increase the mutant frequency in this assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Nestmann E.R., and Lee, E.G.-H. (1983) Mutagenicity of constituents of pulp and paper mill effluent in growing cells of Saccharomyces cerevisiae. Mutat Res 119:273-280.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 98.9%
Method/guideline	L5178Y mouse lymphoma assay (Clive and Spector, 1975; Clive *et al*., 1979)
Test Type	Forward mutation assay
System of Testing	Mammalian
GLP	No
Year	1995
Species/Strain	L5178Y mouse lymphoma
Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced male Sprague-Dawley rat
Doses/Concentration	Without S9: 48, 52, 56, 60, 68, 72, 76, 80, or 84 ug/mL With S9: 20, 24, 28, 32, 36, 40, 48, 56, 64, or 72 ug/mL
Statistical Methods	To be considered positive, *trans*-anethole must have caused a positive dose response and 1 or more of the 3 highest concentrations in the 10% or greater "total growth" range must have exhibited a mutant frequency >2X the background level.
Remarks for Test Conditions	DMSO was used as the vehicle control. Positive control for treatments with S9 was DMBA and without S9 was EMS. Treatments were conducted in triplicate. Mutant frequency results are expressed as an average of 3 plates.
Results	The mutant frequency without S9 for DMSO control 1, DMSO control 2, 48, 52, 56, 60, 68, 72, 76, 80, or 84 ug/mL, 292 ug EMS/mL and 584 ug EMS/mL was 34, 42, 43, 39, 44, 33, 46,

	EMS/mL and 584 ug EMS/mL was 34, 42, 43, 39, 44, 33, 46, 50, 30, 54, 68, 487, and 1,008, respectively. The mutant frequency with S9 for DMSO control 1, DMSO control 2, 20, 24, 28, 32, 36, 40, 48, 56, 64, or 72 ug/mL, 2.5 ug DMBA/mL, and 5.0 ug DMBA/mL was 46, 39, 80, 90, 123, 166, 194, 186, 269, 301, 436, 426, 190, and 534, respectively.
Cytotoxic concentration	Not given
Genotoxic Effects	Without S9 no significant increase in mutant frequency was reported. With S9, a concentration-dependent increase in mutant frequency occurred and was paralleled by a decrease in total growth.
Remarks for Results	Mutant colony size followed a bimodal distribution in the assay with S9 and included a preponderance of small colony mutants.
Conclusion Remarks	With metabolic activation, *trans*-anethole increased the mutant frequency of mouse lymphoma cells in this assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Gorelick N.J. (1995) Genotoxicity of *trans*-anethole in vitro. Mutat Res 326:199-209.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 98%
Test Type	Chromosomal Aberration assay
System of Testing	Mammalian
GLP	No
Year	1995
Species/Strain	Hamster/Chinese ovary cells
Metabolic Activation	S9 fraction from liver of Aroclor 1254-induced male Sprague-Dawley rat
Doses/Concentration	Without S9: 0.025, 0.05, 0.1, or 0.2 ul/ml With S9: 0.013, 0.025, 0.05, or 0.1 ul/ml
Statistical Methods	Fisher's exact test (p less than 0.05)
Remarks for Test Conditions	Without S9, cells were exposed for 18 hours to *trans*-anethole dissolved in DMSO and harvested at 20 h from beginning of treatment. With S9, cells were exposed for 2 hours to *trans*-anethole dissolved in DMSO and harvested at 12 hours from beginning of treatment. One hundred cells were examined per treatment. Exposure concentrations used produced a minimum of 50% reduction in the mitotic index relative to solvent controls. DMSO was used as the vehicle control. For positive controls, triethylenemelamine (TEM) and cyclophosphamide (CP) were

	triethylenemelamine (TEM) and cyclophosphamide (CP) were used for non-S9 and S9-activated cultures, respectively.
Results	For non-activated cells exposed to DMSO, 0.025, 0.05, 0.1, or 0.2 ul/ml or TEM, the mean aberrations/cell were 0.010, 0.040, 0.060, 0.000, 0.026, or 0.400, respectively. For S9-activated cultures exposed to DMSO, 0.013, 0.025, 0.05, or 0.1 ul/ml, or CP, the mean aberrations/cell were 0.010, 0.010, 0.010, 0.040, 0.020, or 0.380, respectively.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	*trans*-Anethole produced no significant increase in the percentage of cells with chromosomal aberrations with or without metabolic activation.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Gorelick N.J. (1995) Genotoxicity of *trans*-anethole in vitro. Mutat Res 326:199-209.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Unscheduled DNA synthesis
Test Type	DNA damage
System of Testing	Mammalian
GLP	No
Year	1992
Species/Strain	Rat/Fisher 344 male hepatocytes
Doses/Concentration	10E-6 to 10E-2 M
Statistical Methods	Ratios (expressed in proportion of control values) of 2 and higher were considered positive
Remarks for Test Conditions	Isolated hepatocytes were seeded at 6.7x10E6 viable cells/90 mm dish and incubated at 37 deg C. The media was changed after 4 hours and 1 hour later, 5 uCi 3H-thymidine (26 Ci/mmol) and 40 ul DMSO (vehicle control) or anethole were added. Cells were incubated a further 20 hours then harvested and the DNA was extracted. Using a Packard liquid scintillation spectrophotometer, 3H-thymidine incorporation was measured (calculated per ug DNA) and the DNA was quantitated using a fluorimetric assay. In addition, separate cultures containing anethole were also tested with 5x10E-9 M 4-fluorochalcone oxide (a cytosolic epoxide inhibitor) or 2.5x10E-3 M L-bethionine-S,R-malfoximine (a glutathione synthesis inhibitor).

Results	Anethole did not produce UDS at any concentration tested. The additon of 4-fluorochalcone oxide or L-bethionine-S,R-malfoximine had no effect on the UDS response.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	Anethole was not genotoxic in the UDS assay.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Caldwell J., Chan, V.S.W., Marshall, A.D., Hasheminejad, G., and Bounds, S.V.J. (1992) 1'-Hydroxylation is the only metabolic pathway of simple alkenylbenzenes involved in their genotoxicity. The Toxicologist 12:56.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Unscheduled DNA synthesis
Test Type	DNA damage
System of Testing	Mammalian
GLP	No
Year	1990
Species/Strain	Rat/Fisher 344 male hepatocytes
Doses/Concentration	10E-6 to 10E-2 M
Remarks for Test Conditions	Isolated hepatocytes were seeded at 6.7x10E6 viable cells/90 mm dish and incubated at 37 deg C. The media was changed after 4 hours and 1 hour later, 5 uCi 3H-thymidine (26 Ci/mmol) and 40 ul DMSO (vehicle control) or anethole were added. Treatments were conducted in duplicate. Cells were incubated a further 16 hours then harvested and the DNA was extracted. Using a Packard Minaxi liquid scintillation spectrophotometer, cells were counted for radioactivity and the DNA was quantitated using a fluorimetric assay. 3H-Thymidine incorporation was calculated per ug DNA. Overnight cytoplasmic lactate dehydrogenase (LDH) leakage was used to determine cell viability. LDH leakage (%) was calculated from LDH (in IU) in the culture medium divided by the total LDH (LDH in culture medium plus cell lysate) x 100.
Results	No UDS was reported. At concentrations >10E-3 M, anethole caused pronounced (Greater than 50%) LDH leakage.
Cytotoxic concentration	10E-3 M
Genotoxic Effects	None

Conclusion Remarks	Anethole was not genotoxic in the UDS assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Howes A.J., Chan, V.S.W., and Caldwell, J. (1990) Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. Fd Chem Toxic 28(8):537-542.

Substance Name	*trans*-Anethole
CAS No.	104-46-1
Method/guideline	Unscheduled DNA synthesis
Test Type	DNA damage
System of Testing	Mammalian
GLP	No
Year	1996
Species/Strain	Rat/SD-CD male and female hepatocytes
Doses/Concentration	10E-6 to 10E-2 M
Remarks for Test Conditions	Isolated hepatocytes were seeded and DMSO (vehicle control) or anethole were added. Cells were incubated, harvested and the DNA was extracted. Using a Packard Minaxi liquid scintillation spectrophotometer, 3H-thymidine incorporation was measured. DNA was quantitated using a fluorimetric assay. UDS was expressed as the ratio of 3H-thymidine incorporation into DNA of treated and control cells. Overnight cytoplasmic lactate dehydrogenase (LDH) leakage was used to determine cell viability. LDH leakage (%) was calculated from LDH (in IU) in the culture medium divided by the total LDH (LDH in culture medium plus cell lysate) x 100.
Results	No effect on UDS. At concentrations greater than 10E-3 M, anethole caused pronounced (greater than 50%) LDH leakage.
Cytotoxic concentration	Greater than 10E-3 M
Genotoxic Effects	None
Conclusion Remarks	*trans*-Anethole was not genotoxic in the UDS assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Marshall A.D., and Caldwell, J. (1996) Lack of influence of modulators of epoxide metabolism on the genotoxicity of *trans*-anethole in freshly isolated rat hepatocytes assessed with the unscheduled DNA synthesis assay. Fd Chem Toxicol 34:337-

unscheduled DNA synthesis assay. Fd Chem Toxicol 34:337-345.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Unscheduled DNA synthesis
Test Type	DNA damage
System of Testing	Mammalian
GLP	No
Year	1989
Species/Strain	Rat/CD or Fisher 344 male hepatocytes
Doses/Concentration	10E-6 to 10E-2 M
Remarks for Test Conditions	Isolated hepatocytes were cultured for 5 hours and then treated with DMSO (vehicle control) or anethole. UDS was assessed by measuring incorporation of 3H-thymidine into isolated DNA. Lactate dehydrogenase (LDH) leakage was calculated by measuring enzyme activity in the culture medium and in the harvested cells after lysis.
Results	No UDS was produced by anethole in either strain of rat. The maximum response was a 1.14-fold increase over control.
Cytotoxic concentration	Non-cytotoxic
Genotoxic Effects	None
Conclusion Remarks	Anethole was not genotoxic in the UDS assay.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Marshall A.D., Howes, A.J., Caldwell, J. (1989) Cytotoxicity and genotoxicity of the food flavour anethole in cultured rat hepatocytes. Hum Toxicol 8(5):404.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Unscheduled DNA synthesis (Williams, 1977, 1980 and Butterworth *et al*., 1987)
Test Type	DNA damage
System of Testing	Mammalian
GLP	No

Year	1989
Species/Strain	Rat/Fischer or Sprague-Dawley hepatocytes
Doses/Concentration	30 ug/ml
Remarks for Test Conditions	Rat hepatocytes were incubated in culture dishes for 18-20 hours with benzaldehyde. Concurrent cell counting or measurement of LDH release was used to determine relative cell survival. UDS was measured by electronically counting nuclear grains and calculating the net nuclear grain count (NNG). At each test concentration, 75-150 cells were analyzed. An increase in NNG of "at least 6 grains per nucleus above the concurrent solvent control value and/or an increase in the percent of nuclei having 6 or more net grains to at least 10% above the concurrent negative control" was considered a positive UDS response.
Results	No genotoxic effects were observed.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	*trans*-Anethole treatment did not increase UDS compared to controls.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Heck J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B. and Curren, R.D. (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1) 1989.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Unscheduled DNA synthesis
Test Type	DNA damage
System of Testing	Mammalian
GLP	No
Year	1994
Species/Strain	Rat/ Wistar hepatocytes
Doses/Concentration	Up to 10E-2 M
Remarks for Test Conditions	Isolated hepatocytes were seeded at 2x10E5 viable cells/25 mm round collagen-coated coverslips. After 2 hours, non-attached cells were removed and DMSO (vehicle control) or anethole were added. 5 uCi 3H-thymidine was added to the media. Cells were harvested after 18 hours of culture and 50

	media. Cells were harvested after 18 hours of culture and 50 hepatocytes per slide from 3 parallel cultures/concentration were evaluated for UDS by counting grains under microscope and determining net grain values (difference between nuclear grain counts and the mean of 3 cytoplasm grain counts). Results were confirmed in an independent repeat study. 2-Acetylaminofluorene (AAF) was used as a positive control.
Results	Net grains showed a slight increase (~2.5 grains) at 10E-3 M (graphically shown), but was cytotoxic at 10E-2 M. Net grains for AAF, DMSO, 10E-5 M, and 10E-4 M were ~26, ~-2.5, 0, and 0.
Cytotoxic concentration	10E-2 M
Genotoxic Effects	Slight increase in UDS.
Conclusion Remarks	*trans*-Anethole was reported to produce a slight increase in UDS at a concentration of 10E-3 M. Lower concentrations did not show any effect.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Mueller L., Kasper, P., Mueller-Tegethoff, K., and Petr, T. (1994) The genotoxic potential in vitro and in vivo of the allyl benzene etheric oils estragole, basil oil and *trans*-anethole. Mutat Res 325:129-136.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Remarks for Substance	Purity 98.9%
Method/guideline	Kada *et al*. (1980)
Test Type	DNA repair test
System of Testing	Bacterial
GLP	No
Year	1982
Species/Strain	*Bacillus subtilis* H17 Rec+ and M45 Rec-
Metabolic Activation	S9 prepared from PCB-treated male Sprague-Dawley rat liver
Doses/Concentration	10 mg/disk
Statistical Methods	Difference between Rec- and Rec+ that was greater than 4mm considered to be evidence of preferential killing of Rec- cells.
Remarks for Test Conditions	Anethole was dissolved in ethanol prior to being pipetted onto sterile 8 mm filter paper disks, which were then placed on agar plates containing 2E5 spores of H17 Rec+ or M45 Rec-. Plates were incubated for 20-24 hours at 37 deg C. Zones of killing (diameter of growth inhibition zone minus diameter of disk) of

	(diameter of growth inhibition zone minus diameter of disk) of both strains were measured. The rec effect was the difference between the strains. Three replicates were performed.
Results	Tests with S9 were not successful. In tests without S9, the mean zone of killing was 5.1 and 2.0 mm for M45 Rec- and H17 Rec+, respectively, resulting in a difference of 3.1 mm.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Conclusion Remarks	Anethole produced negative results in the *Bacillus subtilis* DNA repair test.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Sekizawa J. and Shibamoto, T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutat Res 101:127-140.

4.2.2 *In vivo* Genotoxicity

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Micronucleus assay
Test Type	Clastogenic test
GLP	No
Year	1995
Species/Strain	Mouse/Swiss albino
Sex	Male
Route of Administration	Not specified
Doses/Concentration	250, 500 or 1,000 mg/kg bw/day
Exposure Period	7 days
Remarks for Test Conditions	Cyclophosphamide was used as the positive control and distilled water was used as the vehicle control. Femoral cells were examined.
Appropriate statistical evaluations?	Yes. Student's t-test
Effect on mitotic index or PCE/NCE ratio by dose level and sex	For vehicle control, positive control, 250, 500 and 1,000 mg/kg bw/day, the mean PCE/NCE ratios were 1.03, 0.77, 0.90, 0.84, and 0.82.

and sex	and 0.82.
Genotoxic effects	None
NOEL (C)/ LOEL (C)	NOEL=1,000 mg/kg bw/day
Remarks for Results	Data were presented in tabular form. Although not stated, the route of administration was assumed to be by gavage since this study was part of a larger study in which mice were gavaged with anethole.
Conclusion Remarks	Anethole was non-clastogenic in this study.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Al-Harbi M.M., Qureshi, S., Raza, M., Ahmed, M.M., Giangreco, A.B., and Shah, A.H. (1995) Influence of anethole treatment on the tumour induced by Ehrlick ascites carcinoma in paw of Swiss albino mice. Eur J Cancer Prevent 4:307-318.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Unscheduled DNA synthesis
Test Type	DNA damage
GLP	No
Year	1996
Species/Strain	SD-CD rat
Sex	Female
Route of Administration	Oral-Gavage
Doses/Concentration	0, 1, 125, or 500 mg/kg bw
Exposure Period	Single dose
Remarks for Test Conditions	After a 5-hour fast, groups of female SD-CD rats were gavaged with 0, 1, 125, or 500 mg *trans*-anethole/kg bw in trioctanoin. Positive control animals were gavaged with 500 mg methyleugenol/kg bw and vehicle control animals were gavaged with trioctanoin only. Sixteen hours following treatment, rats were anaesthetized and hepatocytes were isolated and seeded. DMSO (vehicle control) or anethole were added to the cultures. Cells were incubated, harvested and the DNA was extracted. Using a Packard Minaxi liquid scintillation spectrophotometer, 3H-thymidine incorporation was measured. DNA was quantitated using a fluorimetric assay. UDS was expressed as the ratio of 3H-thymidine incorporation into DNA of treated and control cells. Ex vivo positive controls consisted of 2-acetylaminofluorene (AAF) and methyleugenol.

	of 2-acetylaminofluorene (AAF) and methyleugenol.
NOEL (C)/ LOEL (C)	500 mg/kg bw (highest dose tested)
Genotoxic effects	None
Remarks for Results	No UDS response was observed in hepatocytes derived from female rats gavaged with up to 500 mg *trans*-anethole/kg bw. Rats gavaged with methyleugenol did show an increase in UDS.
Conclusion Remarks	*trans*-Anethole was not genotoxic in the UDS assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Marshall A.D., and Caldwell, J. (1996) Lack of influence of modulators of epoxide metabolism on the genotoxicity of *trans*-anethole in freshly isolated rat hepatocytes assessed with the unscheduled DNA synthesis assay. Fd Chem Toxicol 34:337-345.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation
GLP	No
Year	1984
Species/Strain	Mouse/CD-1
Sex	Female
Route of Administration	Intraperitoneal
Doses/Concentration	10 mg/mouse
Exposure Period	Single dose
Remarks for Test Conditions	Groups of 3-4 female CD-1 mice were given an intraperitoneal injection of 0 or 10 mg anethole/mouse in 0.1 ml trioctanoin. Twenty-four hours following treatment, mice were killed and livers were collected and frozen at -80 deg C. DNA was isolated from the frozen livers using a rapid solvent-extraction procedure and quantitated spectrophotometrically. DNA was digested and 32P-labelled. Labeled adducts were purified by reversed phase thin layer chromatography and contact *trans*fer to polyethyleneimine-cellulose. Adduct levels (as reactive adduct labelling [RAL]) were determined (adduct spot/normal nucleotidesx600) and covalent binding indices (CBI) were calculated (umol of anethole bound/mol of DNA nucleotides divided by mmol of anethole administered/kg bw).

	divided by mmol of anethole administered/kg bw).
Remarks for Results	DNA adducts were detected, but showed the least binding when compared with other alkenylbenzenes. The RALx10E7 for Spot 1 and Spot 2 was 3.1 and 1.3, respectively. The CBI was 0.16.
Genotoxic effects	Minimal
Conclusion Remarks	Anethole showed low binding potential to mouse-liver DNA.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Randerath K., Haglund, R.E., Phillips, D.H., and Reddy, M.V. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis 5(12):1613-1622.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation
GLP	No
Year	1984
Species/Strain	Mouse/B6C3F1
Sex	Male
Route of Administration	Intraperitoneal
Doses/Concentration	0.25, 0.5, 1.0, and 3.0 umol
Exposure Period	Postpartum days 1,8, 15, and 22, respectively
Remarks for Test Conditions	Newborn male B6C3F1 mice were given intraperitoneal injections of 0.25, 0.5, 1.0, and 3.0 umole anethole/mouse in trioctanoin on postpartum days 1, 8, 15, and 22, respectively. Mice were weaned on day 28. Groups of 3 mice were killed on days 23, 29, and 43 (i.e., 1, 7, and 21 days after final injection). Livers were collected and pooled. DNA was isolated using a rapid solvent-extraction procedure and quantitated spectrophotometrically. DNA was digested and 32P-labelled. Labelled adducts were purified by reversed phase thin layer chromatography and contact *trans*fer to polyethyleneimine-cellulose. Adducts were detected by autoradiography and radioactivity was measured in a scintillation counter.
Genotoxic effects	Minimal

Remarks for Results	Single adduct spots were detected. Very low levels of DNA binding occurred at 23 days with none detected at 29 or 43 days.
Conclusion Remarks	Anethole produced only low levels of DNA adducts in newborn mice.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Phillips D.H., Reddy, M.V., and Randerath, K. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. II. Newborn male B6C3F1 mice. Carcinogenesis 5(12), 1623-1628.

4.3 Repeated Dose Toxicity

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
GLP	No
Year	1983
Species/strain	Mouse/CD-1
Sex	Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0.46% in the diet
Exposure Period	12 months
Frequency of Treatment	Not specified, assume daily
Control Group	Basal diet
Post Exposure	6 months
Remarks for Test Conditions	Groups of 30 female mice were fed 0.46% *trans*-anethole in the diet either with or without concurrent exposure to 0.05% phenobarbital in drinking water. Anethole exposure was stopped at 12 months. Mice were killed after 18 months and examined for induction of hepatomas.
NOAEL(NOEL)	0.46% (highest dose tested)
Toxic Response/effects by Dose Level	No phenobarbital: for treated mice and controls, respectively, the average number of hepatomas/mouse: 0 and 0. Concurrent phenobarbital: for treated mice and controls, respectively, the average number of hepatomas/mouse: 0.03 and 0.13.

	and 0.13. Mice fed *trans*-anethole showed reduced body weight gain.
Appropriate statistical evaluations?	Yes, Fisher's exact test, Mann-Whitney test
Remarks for Results	No statistically significant change in the average number of hepatomas/mouse compared to control values regardless of exposure to phenobarbital.
Conclusion Remarks	*trans*-Anethole showed no hepatocarcinogenic activity when fed to mice over 12 months.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Well-documented study published in a peer-reviewed journal.
References	Miller E.C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A., and Miller, J.A. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res 43:1124-1134.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
GLP	No
Year	1983
Species/strain	Mouse/A/J
Sex	Female
Route of Administration	Intraperitoneal
Doses/concentration Levels	1 mmol/kg bw
Exposure Period	12 weeks
Frequency of Treatment	Twice per week
Control Group	Vehicle control (trioctanoin) and non-injected
Post Exposure	5 months
Remarks for Test Conditions	Seventeen female mice were given 1 mmol *trans*-anethole/kg bw in trioctanoin by intraperitoneal injection, twice/week for a total of 24 injections. Mice were killed 8 months after the 1st injection and examined for the development of lung adenomas.
NOAEL(NOEL)	1 mmol/kg bw (only dose tested)
Toxic Response/effects by Dose Level	For treated mice, vehicle controls and non-injected controls, respectively, the percent of mice with adenomas: 18, 13, and 4; and the average number of adenomas/mouse: 0.24, 0.13, and 0.04.

Appropriate statistical evaluations?	Yes, Fisher's exact test, Mann-Whitney test
Remarks for Results	No statistically significant change in the percent of mice with lung adenomas or the average number of adenomas/mouse compared to control values.
Conclusion Remarks	*trans*-Anethole showed no pulmonary carcinogenic activity when administered to mice over 12 weeks.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Well documented study published in a peer-reviewed journal.
References	Miller E.C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A., and Miller, J.A. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res 43:1124-1134.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Induction of hepatic microsomal enzymes (Lowry *et al*., 1951; Omura and Sato, 1964; Gibson and Skett, 1986; Lake, 1987)
GLP	No
Year	1992
Species/strain	Rat/Sprague-Dawley CD
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0, 0.25, 0.5 or 1.0% in the diet
Exposure Period	21 days
Frequency of Treatment	Daily
Control Group	Basal diet
Post Exposure	14 days
Remarks for Test Conditions	Groups of 8 rats/sex were fed 0, 0.25, 0.5 or 1.0% *trans*-anethole in the diet for 21 days. Additional groups of 5 rats/sex were treated as above, but were allowed to resume the untreated basal diet for 14 days after the 21-day treatment period. After the treatment period, rats were killed, livers were removed and weighed, and hepatic microsomes were prepared. Hepatic microsomal protein and cytochrome P450 levels were determined using cytochrome C reductase, ethoxycoumarin O-deethylase, ethoxy and pentoxyresurufin O-dealkylase activities. In a supplementary study to determine whether biochemical changes are associated with cell proliferation, groups of 5

	changes are associated with cell proliferation, groups of 5 rats/sex were fed 0, 0.25, 0.5 or 1.0% *trans*-anethole in the diet for 21 days. For the last 3 days of *trans*-anethole exposure, 3 rats/sex/group were given 20 ug 5-bromo-2'-deoxyuridine (BrdU) subcutaneous via osmotic mini-pumps. Rats were killed and livers were removed. Liver sections were taken and treated with a murine anti-BrdU mAb plus a peroxidase-conjugated second antibody.
Toxic Response/effects by Dose Level	For the rats killed on day 22: Corresponding increases in mean protein levels (mg/g liver) for females and males were 8, 15, and 27%, and 13, 17, and 29% over controls, respectively. Corresponding cytochrome P450 contents (nmol/mg protein) for females and males were: 20, 42, and 69%, and 5, 23, and 28% over controls, respectively. These increases were significant (P less than or equal to 0.05) at all doses in females and at the 2 highest doses in males. Significant (P less than or equal to 0.05) increases in relative liver weight were reported in females and males at the 2 highest doses (female, 16 and 42%; male, 13 and 27% over controls). For the rats undergoing a 14-day recovery period, there were no significant differences to controls with the exception of one (considered to be anomalous and related to the lack of sensitivity of the assay) finding of increased cytochrome P450 activity in 0.25% females. In the supplementary study, preliminary data indicate that liver sections from female rats in the 0.5% contain higher numbers of labeled cells than control or 0.25% rats and 1.0% female rats appear to have fewer labeled cells than the other dose groups. No significant changes reported for males.
Appropriate statistical evaluations?	Yes, Student's t-test
Conclusion Remarks	The authors concluded that *trans*-anethole has a modest enzyme-inducing effect on rat liver and noted that female rats tend to be more sensitive. In addition, these effects were reversible when anethole exposure was terminated.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Reed P.M. and Caldwell, J. (1992a) Induction of cytochrome P450 and related enzyme activities following dietary administration of *trans*-anethole to Sprague-Dawley CD rats. Hum Exp Toxicol 11(6):580-581. Reed, P.M. and Caldwell, J. (1992b) Effects of dietary administration of *trans*-anethole on the liver of the Sprague-Dawley CD rat. Toxicol Lett Suppl:283. Presented at the 6th International Congress of Toxicology, Rome, 1992. Reed, P.M. (1994) Hepatocellular changes induced by *trans*-anethole in rodents. A Thesis submitted for the Degree of Doctor of Philosophy in the University of London. Dated March 1994.

Substance Name	*trans*-Anethole
Method/guideline	Immunomodulatory screening test (*Listeria* Challenge)
GLP	Yes
Year	1995
Species/strain	Mouse/B6C3F1
Sex	Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	188, 375, or 750 mg/kg bw/day
Exposure Period	5 days
Frequency of Treatment	Daily
Control Group	Vehicle control (corn oil)
Post Exposure	10 days
Remarks for Test Conditions	Groups of 20 female mice were gavaged with 0,188, 375, or 750 mg *trans*-anethole/kg bw/day for 5 days. On the third day of treatment, mice were also injected by intravenous with *Listeria monocytogenes* (0.2 ml of 1.40x10E6 colony-forming units). Mortality was monitored for 10 days.
Actual dose received by dose level and sex	188, 375, or 750 mg/kg bw/day
Toxic Response/effects by Dose Level	For control, 188, 375, and 750 mg/kg bw/day, total deaths during 10-day observation period were 3/20, 2/20, 1/19, and 3/20, respectively.
Appropriate statistical evaluations?	Yes, Product-Limit Survival Analysis, chi-square test
Remarks for Results	No statistically significant differences in mortality or time to death in treated mice compared to controls.
Conclusion Remarks	*trans*-Anethole did not affect the ability of mice to withstand a Listeria challenge.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	IIT Research Institute (1995a) Immunomodulatory screening test of [*trans*-anethole] administered orally via gavage to B6C3F1 mice. Unpublished report.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Immunomodulatory screening test (Plaque-Forming Cell response to SRBC)

	response to SRBC)
GLP	Yes
Year	1995
Species/strain	Mouse/B6C3F1
Sex	Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	188, 375, or 750 mg/kg bw/day
Exposure Period	5 days
Frequency of Treatment	Daily
Control Group	Vehicle control (corn oil)
Post Exposure	4 days
Remarks for Test Conditions	Groups of 10 female mice were gavaged with 0,188, 375, or 750 mg *trans*-anethole/kg bw/day for 5 days. Four days prior to *trans*-anethole treatment, mice were injected intraperitoneally with 2x10E8 sheep red blood cells (SRBCs) and again after the 5 days of *trans*-anethole treatment. Positive control mice were injected with cyclophosphamide 24 hours prior to assay. Four days after last intraperitoneal injection, mice were killed and spleens were removed. Single cell suspensions were prepared and spleen cell viability was determined. Plaque-forming cells (PFC) were determined from diluted spleen cells incubated for 1 hour in PFC chambers at 37 deg C using a plaque viewer.
Actual dose received by dose level and sex	188, 375, or 750 mg/kg bw/day
Toxic Response/effects by Dose Level	Statistically significant decrease in absolute thymus weight was reported in mice given 750 mg *trans*-anethole/kg bw/day when compared to controls.
Appropriate statistical evaluations?	Yes, ANOVA, Dunnett's test
Remarks for Results	No statistically significant differences in final body weight, absolute and relative spleen weight, relative thymus weight, PFC/10E6 viable cells, viable cells/spleenX10E7, percent cell viability, or PFC/spleen in treated mice compared to controls.
Conclusion Remarks	*trans*-Anethole did not affect the ability of mice to generate antibody plaque-forming cells following immunization with sheep red blood cells.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	IIT Research Institute (1995b) Immunomodulatory screening test of [*trans*-anethole] administered orally via gavage to B6C3F1 mice. Unpublished report.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Immunosuppressive assay
GLP	No
Year	1982
Species/strain	Mouse/B6C3F1
Sex	Male
Route of Administration	Oral-Gavage
Doses/concentration Levels	875 mg/kg bw/day in 1% methylcellulose
Exposure Period	11 days
Frequency of Treatment	Daily
Control Group	Vehicle control (1% methylcellulose)
Post Exposure	None
Remarks for Test Conditions	Groups of 8 male mice were gavaged with 875 mg anethole/kg bw/day for 11 days. On the 3rd day of treatment, mice were intraperitoneally injected with 0.3 ml 25% sheep red blood cells (SRBC). On day 12 of the study, the mice were killed and the spleen, thymus and adrenals were removed and weighed. Serum was also isolated from clotted blood and tested for hemaglutinating activity to SRBC. The antibody index was calculated.
NOAEL(NOEL)	875 mg/kg bw/day (only dose tested)
Actual dose received by dose level and sex	875 mg/kg bw/day
Toxic Response/effects by Dose Level	There were no differences in spleen, thymus and adrenal organ weights or in the agglutination scores and calculated antibody index when compared with control values.
Conclusion Remarks	Anethole was not immunosuppressive in this assay.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Borriston Laboratories, Inc. (1982) Evaluation of immunosuppression in B6C3F1 mice with [anethole]. Final Report dated July 28, 1982.

Substance Name	Anethole (isomer unspecified)

CAS No.	104-46-1
Method/guideline	Induction of hepatic microsomal enzymes
GLP	Yes
Year	1993
Species/strain	Rat/Sprague-Dawley
Sex	Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 75, or 300 mg/kg bw/day in corn oil
Exposure Period	4 days
Frequency of Treatment	Daily
Control Group	Vehicle control (corn oil)
Post Exposure	None
Remarks for Test Conditions	Groups of 7 female rats were gavaged with 0, 75, or 300 mg anethole/kg bw/day in corn oil for 4 days. On the 5th day, body weights were taken, rats were killed and livers were removed and homogenized. The homogenate was centrifuged and the supernatant (S9) was used to determine P450 and P448 activity. Positive controls consisted of S9 from Aroclor 1254-induced rats. Enzyme activity was determined using 3 assays: p-nitroanisole O-demethylation (PNAS), 7-ethoxycoumarin O-deethylation (7EC), and ethoxyresorufin O-deethylation (EROD). The activity of PNAS was determined spectrophotometrically; whereas the activities of 7EC and EROD were determined fluorometrically. Activity was expressed as product formed/mg microsomal protein/hour incubation.
NOAEL(NOEL)	75 mg/kg bw/day
LOAEL(LOEL)	300 mg/kg bw/day
Actual dose received by dose level and sex	0, 75, or 300 mg/kg bw/day
Toxic Response/effects by Dose Level	There were no statistically significant differences in body weight or absolute and relative liver weight in treated rats compared to controls. The enzyme activities (in nmole/mg protein/hr) for control, 75 and 300 mg/kg bw/day, respectively, were: PNAS: 17.6, 20.8, and 36.7 7EC: 137.8, 127.2, and 148 EROD: 0.62, 0.77, and 1.23 The activities were statistically significant for PNAS and EROD at 300 mg/kg bw/day.
Appropriate statistical evaluations?	Yes, ANOVA, Tukey test, Kruskal-Wallis H-test

evaluations?	
Conclusion Remarks	In this assay, anethole induced cytochrome P450 and P448 hepatic activity in rats.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Wenk M.L. (1994) Induction of hepatic microsomal enzymes in rats by [anethole]. Microbiological Associates, Inc. Unpublished report.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Screening method used by U.S. Food and Drug Administration.
GLP	No
Year	1967
Species/strain	Rat/Osborne-Mendel
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	10,000 ppm
Exposure Period	15 weeks
Frequency of Treatment	Daily
Control Group	Basal diet
Post Exposure	None
Remarks for Test Conditions	Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0 or 10,000 ppm for 15 weeks. No vehicle was used. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobin and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological examinations were performed on the liver, kidneys, spleen, heart, and testes of 6-8 animals (evenly divided by sex) from the high dose and control groups.
LOAEL(LOEL)	10,000 ppm
Toxic Response/effects by Dose Level	Slight hydropic microscopic changes of hepatocytes reported in male rats.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.

Remarks for Data Reliability	Code 2. This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
References	Hagan E.C., Hansen W.H., Fitzhugh O.G., Jenner P.M., Jones W.I., Taylor J.M., Long E.L., Nelson A.A., and Brouwer J.B. (1967) Food flavorings and compounds of related structure. II. Subacute and chronic Toxicity. Food Cosmet Toxicol 5:141-157.

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Method/guideline	Screening method used by U.S. Food and Drug Administration.
GLP	No
Year	1967
Species/strain	Rat/Osborne-Mendel
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	2,500 ppm
Exposure Period	1 year
Frequency of Treatment	Daily
Control Group	Basal diet
Post Exposure	None
Remarks for Test Conditions	Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0 or 2,500 ppm for 1 yr. Corn oil (3%) was added to control and test diet as a binder to reduce evaporation of the flavoring. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological examination was performed on the liver, kidneys, spleen, heart, and testes of 6-8 animals (evenly divided by sex) from the high dose and control groups.
NOAEL(NOEL)	2,500 ppm
Toxic Response/effects by Dose Level	No effects reported.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

	Data are considered reliable.
References	Hagan E.C., Hansen W.H., Fitzhugh O.G., Jenner P.M., Jones W.I., Taylor J.M., Long E.L., Nelson A.A., and Brouwer J.B. (1967) Food flavorings and compounds of related structure. II. Subacute and chronic Toxicity. Fd Cosmet Toxicol 5:141-157.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	28-Day range-finding study
GLP	Yes
Year	1997
Species/strain	Mouse/Crl:CD-1 (ICR)BR albino
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	60, 120, 240, 360, or 500 mg/kg bw/day
Exposure Period	28 days
Frequency of Treatment	12 hours/day
Control Group	Basal diet
Post Exposure	None
Remarks for Test Conditions	In a preliminary dose range-finding study, groups of 5 mice/sex were fed 0, 60, 120, 240, 360, or 500 mg *trans*-anethole/kg bw/day via the diet for a period of 28 days. Test diet concentrations were increased to target levels in a step-wise fashion over a 2-week period because of anticipated poor palatability of diet. Diet concentrations were adjusted 2X weekly to compensate for food consumption and body weight. Animals were observed for clinical signs, and body weight and feed consumption changes. Prior to necropsy, hematology and serum chemistry were evaluated. Necropsies were performed during week 5.
NOAEL(NOEL)	360 mg/kg bw/day (female); 120 mg/kg bw/day (male)
LOAEL(LOEL)	500 mg/kg bw/day (female); 240 mg/kg bw/day (male)
Actual dose received by dose level and sex	Males: 0, 57.8, 115.3, 218.6, 290.7, or 44.2 mg/kg bw/day; females: 0, 59.2, 113.8, 235.4, 348.4, or 454.8 mg/kg bw/day
Toxic Response/effects by Dose Level	60 mg/kg bw/day: no effects 120 mg/kg bw/day: decreased feed consumption 240 mg/kg bw/day:some mice stopped eating and died; 40% mortality in males; decreased body weights in males on day 29 (83.6% of controls)

	360 mg/kg bw/day:some mice stopped eating and died; 60% mortality in males; decreased body weights in males on day 29 (74.8% of controls); significantly lower leukocyte counts 500 mg/kg bw/day:some mice stopped eating and died; 40% mortality in males and females; decreased body weights on day 29 (84.5% and 81% of controls for males and females); significantly lower leukocyte counts No treatment-related histomorphological changes in the liver at any dose.
Appropriate statistical evaluations?	Yes, ANOVA, Dunnett's t-test, Leven's test, Bartlett's test
Remarks for Results	The results from this study appear to be related to the poor palatability of *trans*-anethole in the diet and compromised food intake.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Minnema D.J. (1997b) 28-Day range-finding dietary toxicity study of *trans*-anethole in mice. CHV 2595-101. Corning Hazleton Inc., Unpublished report.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Induction of hepatic microsomal enzymes
GLP	No
Year	1992
Species/strain	Rat/SD-CD
Sex	Female
Route of Administration	Intraperitoneal
Doses/concentration Levels	300 mg/kg bw/day in trioctanoin
Exposure Period	7 days
Frequency of Treatment	Daily
Control Group	Vehicle control (trioctanoin)
Remarks for Test Conditions	Groups of 24 female rats were injected intraperitoneally with 0 or 300 mg *trans*-anethole/kg bw/day for 7 days. Twenty-four hours following last injection, rats were killed, livers were removed and weighed, and hepatic microsomes were prepared. Positive control rats were treated with the known inducers beta-naphthoflavone (BNF), phenobarbitone (PB), or isosafrole (ISF). Cytochrome P450 activity was determined using 7-ethoxycoumarin O-deethylase expressed in nmol/min/mg protein.

	protein.
LOAEL(LOEL)	300 mg/kg bw/day
Actual dose received by dose level and sex	300 mg/kg bw/day
Toxic Response/effects by Dose Level	There was a significant (P less than or equal to 0.05) increase in relative liver weights, microsomal protein expressed as mg/g liver (18% increase) and in microsomal cytochrome P450 expressed as nmol/mg protein (45% increase) in anethole-treated rats compared to vehicle controls. Enzyme activity was reported to be 69, 1580, 290, and 590% greater than vehicle controls for anethole, BNF, PB, and ISF, respectively.
Appropriate statistical evaluations?	Yes, Student's t-test
Conclusion Remarks	The authors concluded that *trans*-anethole has a modest enzyme-inducing effect on rat liver.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Reed P.M. and Caldwell, J. (1992a) Induction of cytochrome P450 and related enzyme activities following dietary administration of *trans*-anethole to Sprague-Dawley CD rats. Hum Exp Toxicol 11(6):580-581.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Induction of hepatic microsomal enzymes
GLP	No
Year	1993
Species/strain	Mouse/CD-1
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0, 0.1, 0.25, 0.5 or 1.0% in the diet
Exposure Period	22 days
Frequency of Treatment	Daily
Control Group	Basal diet
Remarks for Test Conditions	Groups of 24 mice/sex were fed 0, 0.1, 0.25, 0.5 or 1.0% *trans*-anethole in the diet for 22 days. Mice in the 1.0% group were terminated prematurely due to severe weight loss. After the treatment period, mice were killed, livers were removed and weighed, and hepatic microsomes were prepared. Livers were pooled in 3s (i.e., n=8). Hepatic microsomal protein and

	pooled in 3s (i.e., n=8). Hepatic microsomal protein and cytochrome P450 levels were determined.
Toxic Response/effects by Dose Level	The diet was unpalatable to the mice resulting in decreased body weight in the 0.25 and 0.5% groups (body weight decrease for males, 72 and 70% and for females, 81 and 68% of controls). Relative liver weights were significantly (p less than or equal to 0.005) increased at the 2 lowest doses, but not at 0.5% (males, 136, 120, and 106% of controls; females, 117, 115, and 105% of controls). Microsomal protein was significantly (p less than or equal to 0.005 and p less than or equal to 0.05) increased in males given 0.25 and 0.5% (21 and 29% of controls, respectively). Cytochrome P450 was significantly (p less than or equal to 0.005 and p less than or equal to 0.05) increased in males and females of the 0.5% group (113 and 121% of controls, respectively). Females also showed a significant (p less than or equal to 0.05) increase (11%) at 0.25%.
Remarks for Results	Since caloric restriction is known to induce hepatic cytochrome P450, a similar study was conducted using 0.5% *trans*-anethole but restricted the dietary intake of control mice to that consumed by the treated mice. The comparison of microsomal cytochrome P450 content in these mice still showed a significant (p less than or equal to 0.05) increase (33%) over controls.
Conclusion Remarks	The authors concluded that *trans*-anethole has a modest enzyme-inducing effect on mouse liver.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Reed P.M. and Caldwell, J. (1993) The effects of dietary administration of the food flavour *trans*-anethole on mouse liver. Hum Exp Toxicol 12(6):565.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	28-Day range-finding study
GLP	Yes
Year	1997
Species/strain	Rat/Sprague-Dawley Crl:Cd BR
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	150, 300, 600, 900, or 1,200 mg/kg bw/day
Exposure Period	28 days

Frequency of Treatment	12 hours/day
Control Group	Basal diet
Post Exposure	None
Remarks for Test Conditions	In a preliminary dose range-finding study, groups of 5 rats/sex were fed 0, 150, 300, 600, 900, or 1,200 mg *trans*-anethole/kg bw/day via the diet for a period of 28 days. Test diet concentrations were increased to target levels in a step-wise fashion over a 2-week period because of anticipated poor palatability of diet. Diet concentrations were adjusted 2X weekly to compensate for food consumption and body weight. Animals were observed for clinical signs, and body weight and feed consumption changes. Prior to necropsy, hematology and serum chemistry were evaluated. Necropsies were performed during week 5.
NOAEL(NOEL)	NOAEL: 600 mg/kg bw/day (both sexes) NOEL 300 mg/kg bw/day (males), 600 mg/kg bw/day (females)
LOAEL(LOEL)	LOAEL: 1,200 mg/kg bw/day (both sexes) LOEL 600 mg/kg bw/day (males), 1,200 mg/kg bw/day (females)
Actual dose received by dose level and sex	Males: 0, 146.3, 297.8, 597.8, 903.8, or 1,095.9 mg/kg bw/day; females: 0, 148.4, 292.7, 599.3, 882.4, or 1,149.2 mg/kg bw/day
Toxic Response/effects by Dose Level	No notable treatment-related findings at 150 or 300 mg/kg bw/day, although decreased feed consumption was noted in the early part of the study. At 600 mg/kg bw/day: decreased feed consumption was noted in the early part of the study; increased relative brain with stem weight (males only); increased relative liver weight (females only); decreased relative (to brain) kidney and thymus weights (males only); decreased serum triglycerides in males At 900 mg/kg bw/day: decreased feed consumption was noted in the early part of the study, lower terminal body weights in males (82% of controls); decreased absolute kidney and weights (males only); decreased absolute adrenal weight (females only); increased relative brain with stem weight (males only); increased relative liver weight (females only); decreased relative (to brain) adrenal weight (females only); increased gamma-glutamyl*trans*ferase; increased total cholesterol (females only); decreased serum triglycerides in males; decreased inorganic phosphorus values (males only); decreased mean cell volume and mean cell hemoglobin values (females only); cytoplasmic clearing (pallor) of hepatocytes in centrilobular to midzonal regions At 1,200 mg/kg bw/day: decreased feed consumption, lower terminal body weights in males (68% of controls); decreased absolute kidney, liver, and thymus weights (males only); decreased absolute adrenal weight; increased relative brain with stem weight (males only); increased relative liver weight; decreased relative (to brain) kidney and thymus weights (males only); decreased relative (to brain) adrenal weight (females only); increased gamma-glutamyl*trans*ferase, increased alanine amino*trans*ferase (males only) increased total cholesterol (females only); decreased serum triglycerides in males,

	(females only); decreased serum triglycerides in males, decreased inorganic phosphorus values (males only), decreased mean cell volume and mean cell hemoglobin values, cytoplasmic clearing (pallor) of hepatocytes in centrilobular to midzonal regions
Appropriate statistical evaluations?	Yes, ANOVA, Dunnett's t-test, Leven's test, Bartlett's test
Remarks for Results	The decreased values for mean cell volume and mean cell hemoglobin were not accompanied by significant differences in mean erythrocyte count, hemoglobin or hematocrit. Since the decrease was of low magnitude and within reference ranges, the effect could not be definitively attributed to anethole exposure.
Conclusion Remarks	Anethole administered at doses of 900 or 1,200 mg/kg bw/day produced some hepatic effects as shown in serum biochemistry results and microscopic examination.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Minnema D.J. (1997a) 28-Day range-finding dietary toxicity study of *trans*-anethole in rats. CHV 2595-102. Corning Hazleton Inc. Unpublished report.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	90-day dietary study
GLP	Yes
Year	1997
Species/strain	Mouse/Crl:CD-1 (ICR)BR albino
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0, 30, 60, 120, or 240 mg/kg bw/day
Exposure Period	90 days
Frequency of Treatment	12 hours/day
Control Group	Basal diet
Post Exposure	None
Remarks for Test Conditions	In a 90-day study, groups of 20 mice/sex were fed 0, 30, 60, 120, or 240 mg *trans*-anethole/kg bw/day via the diet. Test diet concentrations were increased to target levels in a step-wise fashion over a 2-week period because of anticipated poor palatability of diet. Diets were prepared weekly to compensate for food consumption and body weight. Animals were assessed

	for food consumption and body weight. Animals were assessed by clinical observations, body weight gains, food consumption, food utilization efficiency, ophthalmoscopy exam, hematology and serum chemistry evaluations, gross pathology, and histopathology.
NOAEL(NOEL)	240 mg/kg bw/day (highest dose tested)
Actual dose received by dose level and sex	Males: 0, 29.7, 59.6, 116.8, or 236.0 mg/kg bw/day; females: 0, 30, 60.5, 120.7, or 239.6 mg/kg bw/day
Toxic Response/effects by Dose Level	No treatment-related ophthalmology findings. Reported effects: reduced body weight (at 120 mg/kg bw/day and higher), increased mortality (at 60 mg/kg bw/day and higher in males and 120 mg/kg bw/day and higher in females), decreased feed consumption (at 120 mg/kg bw/day and higher), decreased feed utilization efficiency (at 120 mg/kg bw/day and higher in males), liver glycogen depletion (at 30 mg/kg bw/day and higher in males and 60 mg/kg bw/day and higher in females), decreased mean cell volume (at 120 mg/kg bw/day in males), decreased mean cell hemoglobin (at 120 mg/kg bw/day and higher in males), reduced cellularity of the spleen (at 240 mg/kg bw/day in males), delayed kidney development (at 240 mg/kg bw/day in males), increased absolute and relative liver weights (at 30 mg/kg bw/day and higher in males), increased relative thyroid weight (at 30 mg/kg bw/day and higher in males), decreased absolute spleen weight (at 60 mg/kg bw/day and higher in males), decreased relative (to brain) spleen weight (at 60 mg/kg bw/day and higher in males), decreased absolute and relative (to brain) kidney weights (at 120 mg/kg bw/day and higher in males), increased absolute and relative adrenal weights (at 60 mg/kg bw/day and higher in males), decreased absolute heart and adrenal weights (at 240 mg/kg bw/day in females), increased incidence of centrilobular hepatocellular hypertrophy (at 60 mg/kg bw/day and higher in males), and increased serum alkaline phosphatase (at 120 mg/kg bw/day and higher in males).
Appropriate statistical evaluations?	Yes, ANOVA, Dunnett's t-test, Leven's test, Bartlett's test
Remarks for Results	Severe loss of body weight and dehydration reported mainly at doses of 120 mg/kg bw/day and higher were attributed to inanition syndrome (starved mouse syndrome) resulting from the poor palatability of the diet and reduced food intake. The enlarged livers, increased liver weight, and increased incidence of centrilobular hepatocellular hypertrophy were considered to be adaptive physiological responses. Increased serum alkaline phosphatase was considered to be also an adaptive response, or related to the reduced feed intake. The decreased values for mean cell volume and mean cell hemoglobin were not accompanied by significant differences in mean erythrocyte count, hemoglobin or hematocrit. In addition, the decrease was of low magnitude and therefore, the changes were considered incidental.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.

References	Minnema D.J. (1997c) 90-Day subchronic dietary toxicity study of *trans*-anethole in mice. CHV2595-103. Corning Hazleton Inc. Unpublished report.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Method/guideline	Chronic toxicity/carcinogenicity dietary study
GLP	Ambiguous
Year	1989
Species/strain	Rat/CD-outbred
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0, 0.25, 0.5, or 1.0% in the diet
Exposure Period	Up to 177 weeks
Frequency of Treatment	Daily
Control Group	Basal diet
Post Exposure	10 rats/sex given 1%, stopped treatment at week 54
Remarks for Test Conditions	Groups of 52-78 rats/sex were fed 0, 0.25, 0.5, or 1.0% *trans*-anethole in the diet for up to 177 weeks. An additional group of 26 rats/sex was fed 1% *trans*-anethole until week 54 and then received basal diet only until the end of the study. Animals were assessed using clinical observations, body weight, feed consumption, hematology, and pathology.
NOAEL(NOEL)	0.25%
LOAEL(LOEL)	0.5%
Actual dose received by dose level and sex	Males: 0, 100, 200, or 400 mg/kg bw/day; females: 0, 120, 250, or 550 mg/kg bw/day
Toxic Response/effects by Dose Level	Between weeks 42-45, most rats showed signs of sialodacryoadenitis resulting in *trans*ient retardation of body weight gain. All treated groups showed lower body weight gains. The reversal group showed no difference in body weight gain compared to controls by the end of the study. Mortality was increased in females receiving 1% *trans*-anethole. Reduced adiposity was reported in high-dose rats, particularly males. No effect on hematological parameters. Notable effects on the liver were: sinusoidal dilatation (at 0.5 and 1%); nodular hyperplasia (at 0.5 and 1% in males and 1% in females); and hepatocytic hypertrophy (at 0.5 and 1% in females). The only statistically significant finding in neoplastic lesions was an increase in the incidence of liver tumors in 1% females.

Appropriate statistical evaluations?	Yes, Student's t-test, chi-square test, one-tailed chi-squared test
Remarks for Results	The reduced adiposity was considered to be an indirect effect of the poor palatability of the treated diet and decreased feed consumption. The authors noted that the increased incidence of hepatocellular carcinomas reported in high-dose females were "late onset", had no effect on longevity and was still within the range of historical controls.
Conclusion Remarks	The authors stated that there was insufficient evidence to conclude that *trans*-anethole is a human carcinogenic risk.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Truhaut R., Le Bouhis, B., Attia, M., Glomot, R., Newman, J., and Caldwell, J. (1989) Chronic toxicity/carcinogenicity study of *trans*-anethole in rats. Fd Chem Toxicol 27(1):11-20.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
GLP	No
Year	1983
Species/strain	Mouse/CD-1
Sex	Male
Route of Administration	Intraperitoneal
Doses/concentration Levels	Total dose of 9.45 umol over 4 injections at a ratio of 1:2:4:8
Exposure Period	22 days
Frequency of Treatment	Days 1, 8, 15, and 22 of age
Control Group	Vehicle control (trioctanoin) and non-injected
Post Exposure	12 months
Remarks for Test Conditions	Fifty-three male mice given intraperitoneal injections at 1, 8, 15 and 22 days of age of a total of 9.45 umol *trans*-anethole in a ratio of 1:2:4:8 and given in 0.025, 0.05, 0.05, and 0.1 ml, respectively. Mice were weaned at 22 days of age, killed at 12 months of age and examined for induction of hepatomas.
NOAEL(NOEL)	9.45 umol (only dose tested)
Actual dose received by dose level and sex	Total dose of 9.45 umol
Toxic Response/effects by Dose Level	For treated males, vehicle controls and non-injected controls, respectively, the percent of hepatoma-bearing mice: 33, 26, and 15; the average number of hepatomas/mouse: 0.5, 0.5 and 0.2; and the number of mice with lung adenomas: 2, 2, and 1.

	0.2; and the number of mice with lung adenomas: 2, 2, and 1.
Appropriate statistical evaluations?	Yes, Fisher's exact test, Mann-Whitney test
Remarks for Results	No statistically significant change in the percent of hepatoma-bearing mice, average number of hepatomas/mouse, or number of mice with lung adenomas compared to control values.
Conclusion Remarks	*trans*-Anethole showed no hepatocarcinogenic activity when administered to mice prior to weaning.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Well documented study published in a peer-reviewed journal.
References	Miller E.C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A., and Miller, J.A. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res 43:1124-1134.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
GLP	No
Year	1983
Species/strain	Mouse/CD-1
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	2.5 or 5 mmol/kg bw
Exposure Period	4-5 weeks
Frequency of Treatment	2 times/week
Control Group	Vehicle control (trioctanoin)
Post Exposure	10-13 months
Remarks for Test Conditions	Groups of 55-67 mice/sex were gavaged with 0, 2.5 or 5 mmol *trans*-anethole/kg bw twice weekly for a total of 10 times starting at an age of 4 days. Mice were killed between 11 and 14 months of age and examined for induction of hepatomas.
NOAEL(NOEL)	5 mmol/kg bw (highest dose tested)
Actual dose received by dose level and sex	2.5 or 5 mmol/kg bw
Toxic Response/effects by Dose Level	For 2.5 mmol/kg bw females, 2.5 mmol/kg bw males, 5.0 mmol/kg bw females, 5.0 mmol/kg bw males, vehicle control females and vehicle control males, respectively, the percent of hepatoma-bearing mice: 2, 18, 4, 31, 2, and 24; the average

	hepatoma-bearing mice: 2, 18, 4, 31, 2, and 24; the average number of hepatomas/mouse: 0.02, 0.3, 0.04, 0.6, 0.02, and 0.6; and the number of mice with lung adenomas: 2, 2, 2, 2, 2, and 0.
Appropriate statistical evaluations?	Yes, Fisher's exact test, Mann-Whitney test
Remarks for Results	No statistically significant change in the percent of hepatoma-bearing mice, average number of hepatomas/mouse, or number of mice with lung adenomas compared to control values.
Conclusion Remarks	*trans*-Anethole showed no hepatocarcinogenic activity when administered to mice prior to weaning.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Well documented study published in a peer-reviewed journal.
References	Miller E.C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A., and Miller, J.A. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res 43:1124-1134.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
GLP	No
Year	1983
Species/strain	Mouse/B6C3F1
Sex	Male
Route of Administration	Intraperitoneal
Doses/concentration Levels	Total dose of 4.75 umol over 4 injections at a ratio of 0.6:2:4:12
Exposure Period	22 days
Frequency of Treatment	Days 1, 8, 15, and 22 of age
Control Group	Vehicle control (trioctanoin) and non-injected
Post Exposure	12 months
Remarks for Test Conditions	Fifty male mice given intraperitoneal injections at 1, 8, 15 and 22 days of age of a total of 4.75 umol *trans*-anethole in a ratio of 0.6:2:4:12 and given in 15, 50, 25, and 75 ul, respectively. Originally, the injections were given in a ratio of 1:2:4:12, but 50% of the mice died within the first week and the experiment was redone. Mice were weaned at 4 weeks of age. Fourteen mice were examined by laparotomy at 13 months and those surviving were killed at 18 months of age and examined for induction of hepatomas.

	induction of hepatomas.
NOAEL(NOEL)	4.75 umol (only dose tested)
Actual dose received by dose level and sex	Total dose of 4.75 umol
Toxic Response/effects by Dose Level	Data from laparotomy at 13 months for treated males, vehicle controls and non-injected controls, respectively, the percent of hepatoma-bearing mice: 7, 5, and 12; and the average number or hepatomas/mouse: 0.1, 0.1 and 0.2. Data from at study termination for treated males, vehicle controls and non-injected controls, respectively, the percent of hepatoma-bearing mice: 32, 41, and 28; and the average number of hepatomas/mouse: 0.4, 0.5 and 0.5.
Appropriate statistical evaluations?	Yes, Fisher's exact test, Mann-Whitney test
Remarks for Results	No statistically significant change in the percent of hepatoma-bearing mice, or average number of hepatomas/mouse compared to control values.
Conclusion Remarks	*trans*-Anethole showed no hepatocarcinogenic activity when administered to mice prior to weaning.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Well documented study published in a peer-reviewed journal.
References	Miller E.C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A., and Miller, J.A. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res 43:1124-1134.

4.4 Reproductive Toxicity

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 98%
Method/Guideline	4-Generation reproduction study
Test Type	Reproductive toxicity
GLP	No
Year	1971
Species/Strain	Rat/Wistar SPF
Sex	Male and Female

Route of Administration	Oral-Diet
Duration of Test	4 generations with a minimum exposure to the treated diet of 70 days from time of weaning
Doses/Concentration	1% in the diet (approximately 600-1,500 mg/kb bw/day)
Premating Exposure period for males	F0: 70 days F1-F4: raised on treated diet
Premating Exposure period for females	F0: 70 days F1-F3: raised on treated diet
Control Group and Treatment	Basal diet
Frequency of Treatment	Daily
Remarks for Test Conditions	Groups of 20 male and 20 female Wistar SPF rats were fed 0 or 1% anethole in the diet (approximately 600-1,500 mg/kg bw/day) for 70 days prior to mating. Four paired groups were formed: (1) control males X control females; (2) control males X treated females; (3) treated males X control females; and (4) treated males X treated females. During the mating period of 15 days, the first 3 groups were maintained on basal diet; whereas, group 4 received treated diet. During gestation and lactation, females of groups 2, 3 and 4 were maintained on 1% anethole diet. Offspring from groups 1 and 4 were used for propagating the next generation and were raised on the same dietary treatment as their parents (70 days from time of weaning). At approximately 3 months of age, rats were bred to obtain the next generation. A similar procedure was followed to obtain the 3rd and 4th generations. The treatment groups for F1, F2 and F3 were: (1) control males X control females; and (2) treated males X treated females. Mortality, body weight, food consumption, and reproductive performance (fertility, sex ratio, date of birth, stillbirths, clinical observations, litter size, litter viability) were monitored.
Actual dose received by dose level and sex	Approximately 600 to 1,500 mg/kg bw/day
Parental data and F1 as appropriate	F0: death of 1 control male and 1 treated female, no other deaths, decreased body weight in treated rats, decreased food consumption in treated rats, no effect on reproductive performance. F1: no deaths, reduced body weight gain and body weight in treated rats, reduced food consumption in treated rats for 1st 2 weeks, no effect on reproductive performance.
Offspring toxicity F1 and F2	F2 and F3: no deaths, reduced body weight gain and body weight in treated rats, reduced food consumption in treated rats for first 2 weeks, no effect on reproductive performance
Appropriate statistical evaluations?	Yes, one factor variance analysis, Fischer test, t-test, Chi-square test
Remarks for Results	The reduced palatability of the diet was considered to be responsible for the lower body weight gain and body weights of the rats receiving anethole.

	the rats receiving anethole.
Conclusion remarks	*trans*-Anethole did not affect the reproductive performance of rats over 4 generations.
Data Reliabilities Qualities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Le Bourhis B. (1973b) 4-Generation reproduction study in rats given *trans*-anethole in the diet. Unpublished report by Sophie Holm. Laboratoire de Physiologie, Institut de Recherches appliquees aux Boissons, Montreuil, 93, France.

Substance Name	*trans*-Anethole
CAS No.	4180-23-8
Remarks for Substance	Purity greater than 98%
Method/Guideline	Cross-fostering
Test Type	Reproductive toxicity
GLP	No
Year	1971
Species/Strain	Rat/Wistar SPF
Sex	Male and Female
Route of Administration	Oral-Diet
Duration of Test	1 generation
Doses/Concentration	1% in the diet (approximately 600-1,500 mg/kb bw/day)
Premating Exposure period for males	Control F1 males from 4-generation portion of study
Premating Exposure period for females	Control and treated F1 females from 4-generation portion of study
Control Group and Treatment	Basal diet
Frequency of Treatment	Daily
Remarks for Test Conditions	In a cross-fostering experiment, groups of 6 control and 6 treated F1 females (receiving 1% anethole in the diet) were mated with control F1 males (from 4-generation portion of study). Litters born from treated females were exchanged with litters from control females at birth and reared by the new dams. Body weight and growth of pups was monitored.
Actual dose received by dose level and sex	Approximately 600-1,500 mg/kb bw/day

Parental data and F1 as appropriate	F1: no significant difference in body weights of pups from those nursed by mothers of the same group, regardless from which group they were born; final body weights of pups born from treated dams but raised by control dams regained normal values by day 28
Appropriate statistical evaluations?	Yes, one factor variance analysis, Fischer test, t-test, Chi-square test
Remarks for Results	Reduced palatability of diets containing anethole was considered an issue in the nutritional status of the dams.
Conclusion remarks	The results indicate that postnatal growth is not directly affected by anethole exposure, but is a result of the nutritional status of the dams.
Data Reliabilities Qualities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Le Bourhis B. (1973b) 4-Generation reproduction study in rats given *trans*-anethole in the diet. Unpublished report by Sophie Holm. Laboratoire de Physiologie, Institut de Recherches appliquees aux Boissons, Montreuil, 93, France.

4.5 Developmental/Teratogenicity Toxicity

Substance Name	Anethole (isomer unspecified)
CAS No.	104-46-1
Test Type	Developmental toxicity
GLP	Yes
Year	1992
Species/strain	Rat/Crl:CDBR VAF/Plus Sprague-Dawley
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Approximately 32 days
Doses/concentration Levels	0, 35, 175, or 350 mg/kg bw/day
Exposure Period	Approximately 32 days
Frequency of Treatment	Daily
Control Group and Treatment	Corn oil vehicle
Remarks for Test Conditions	Groups of 10 female rats were gavaged with anethole 0, 35, 175, or 350 mg/kg bw/day in corn oil for 7 days prior to co-habitation with male rats until day 4 of lactation for those rats producing litters and day 25 of cohabitation for those rats

	producing litters and day 25 of cohabitation for those rats without confirmed mating dates. Body weight and feed consumption was monitored. Fertility, gestation index, implantation sites, length of gestation, number of stillborn pups, litter size, pup viability, pup weight, and clinical observations of pups were recorded. On day 4 of lactation, pups were examined, killed, and discarded.
NOAEL(NOEL) maternal toxicity	35 mg/kg bw/day
LOAEL(LOEL) maternal toxicity	175 mg/kg bw/day
NOAEL (NOEL) developmental toxicity	175 mg/kg bw/day
LOAEL (LOEL) developmental toxicity	350 mg/kg bw/day
Actual dose received by dose level and sex	0, 35, 175, or 350 mg/kg bw/day
Maternal data with dose level	At 350 mg/kg bw/day: significantly reduced mean body weight and feed consumption throughout study; 1 rat found dead on day 20 of gestation (necropsy showed congested lungs, but uterine contents showed 17 normal fetuses and 2 early resorptions); 2 rats had urine-stained abdominal fur during the premating period, one of these rats also "had a tan perivaginal substance and appeared pale on day 23 of gestation, and during lactation was emaciated and pale and had an ungroomed coat and red perioral and perivaginal substances"; in necropsy 1 rat had a raised yellow area in the liver, 1 rat had hematomas on the vessels supplying the implantation sites; average gestation duration was increased (number of dams delivering on days 23 and 24 was increased over controls); number of dams with stillborn pups and with all pups dying before postpartum day 4 was significantly increased (P less than or equal to 0.01). At 175 mg/kg bw/day, mean body weight was significantly decreased on gestation days 6 and 14; feed consumption was significantly reduced during premating days 1-8 but not during gestation
Fetal Data with Dose Level	At 350 mg/kg bw/day, number of live born pups (75) was significantly decreased (P less than or equal to 0.01) compared to controls (147), number of stillborn pups (18) was significantly increased (P less than or equal to 0.01) compared to controls (0), number of pups dying on day 1 and days 2-4 (8 and 7 respectively) was significantly increased (P less than or equal to 0.01) compared to controls (0 and 0, respectively), viability index (number of live pups on postpartum day 4/number of live born pups on postpartum day 1) was significantly (P less than or equal to 0.01) decreased (80%) compared to controls (99.3%); number of surviving pups/litter on postpartum day 4 (7.5) was significantly (P less than or equal to 0.01) decreased compared to controls (14.6); live litter size on postpartum day 4 (12.0) was significantly (P less than or equal to 0.05) decreased compared to controls (14.6); pup weight/litter on postpartum day 1 (5.1 g) was significantly (P less than or equal to 0.05) decreased compared to controls (6.2 g).

	decreased compared to controls (6.2 g). No other effects were reported at the other doses. No anomalies were reported.
Appropriate statistical evaluations	Yes, Bartlett's Test, ANOVA, Dunnett's test, Kruskal-Wallis Test, Dunn's test, Fischer's Test
Conclusion Results	Anethole did not cause any developmental effects on the rat fetus at doses below those causing maternal toxicity (reduced body weight and feed consumption).
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Argus Research Laboratories, Inc. (1992) Reproductive and developmental toxicity screening test of (anethole) administered orally via gavage to Crl:CDBR VAF/Plus female rats. Final Report.